CHEMICAL INTERACTIONS OF CITRATE, CALCIUM AND PHOSPHATE IN A CALCAREOUS SASKATCHEWAN SUBSOIL

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in Partial Fulfillment of the Requirements
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ABSTRACT

The addition of citrate into biostimulatory amendment solutions for the in-situ bioremediation of petroleum hydrocarbons is a relatively new concept. Research has shown that addition of citrate in combination with phosphate can potentially decrease P sorption and thus increase P mobility and bioavailability. In this thesis, soil cores from a hydrocarbon-contaminated site were first characterized to determine soil characteristics. The soil cores had a high clay content with heterogeneous soil layers, leading to the hypothesis that preferential flow paths are likely dominant in transport onsite. As a manipulative experiment, soil Ca levels were modified via three treatments, Ca saturated, Ca depleted, and the control soil to ascertain the effect of Ca upon citrate and P retention. X-ray fluorescence (XRF) and x-ray diffraction (XRD) confirmed the change in total Ca levels as well as differences in mineralogy of the treated soils. In chapter three, phosphate adsorption isotherms and citrate desorption isotherm experiments were designed to quantify the relationships among soil Ca, P adsorption, and the concentration of citrate (0-50mM) at equilibrium conditions. The results demonstrated that high citrate levels (above 5mM) would allow less P to be adsorbed onto the soil surface. Phosphorus K-edge XANES were measured on soil samples treated with citrate and Ca to determine the relationship between chemical speciation and citrate interactions. The research led to the discovery that higher than expected (>5mM) levels of citrate were required to get a significant desorption in P; higher levels of soil Ca were correlated with more adsorbed P. In chapter four, kinetics experiments combined with a spectroscopic approach yielded a better understanding of the citrate-phosphate-calcium reaction dynamics. By using the Pseudo Second order model, all parameters from the kinetics experiments were highly correlated to model experimental values. The major finding of the kinetic study was that soil Ca level strongly influenced citrate desorption effectiveness. Overall this thesis showed that the connection between Ca, citrate, and P was important in determining rates and mechanisms of chemical interaction and to be able to build and apply a model to the system.

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DEDICATION

To all those who went on this adventure with me. To my family who stood beside me the whole time.

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LIST OF ABBREVIATIONS

SOC	Soil organic carbon
CEC	Cation exchange capacity
EC	Electro-conductivity
XANES	X-ray Absorption Near Edge Structure
SOM	Soil organic matter
OC	Organic carbon
OM	Organic matter
CLS	Canadian Light Source
LMWOA	Low Molecular Weight Organic Acids
PCA	Principal component analysis

1. INTRODUCTION

Soil contamination of petroleum hydrocarbons (PHC) is a major environmental concern in Canada and around the world, where 60% of Canada's contaminated sites contain PHCs (about 12,000). Hydrocarbon contamination generally occurs through the leaking of petroleum underground storage tanks (UST) located at fuel stations and via spills in bulk transfer stations. Logistics (for urban sites) and cost (for rural sites) often make excavation and ex-situ remediation approach; there is a compelling need to find in-situ remediation techniques that works in low temperature, high clay, and carbonate-rich calcareous soils of the Canadian prairies. In subsurface systems that have been contaminated with petroleum hydrocarbons (PHC), microbial degradation is often limited by nutrient availability, specifically phosphorus (P) (Leahy and Colwell, 1990). Thus, providing an effective source of nutrients to PHC-contaminated soils could greatly increase the rate of in-situ bioremediation and save both time and money to industry. The use of microbes for remediation is not a new practice, however boosting their abilities using different techniques is less studied.

The transport of P to the contaminant plume to stimulate remediation is important, but minimizing P mobilization offsite is equally important since P is of environmental concern in fresh water sources such as lakes, rivers and groundwater (Hsieh et al., 2007). Surface runoff and erosion are considered to be the main pathway for P losses in many systems (Andersson et al., 2013). The geochemistry of the site is highly relevant for nutrient deliveries as our element of concern P is highly hindered in highly calcareous clay rich soils. Understanding fundamentally how Ca interacts with P in these soils will be paramount to this study.

The soils used in this thesis are calcareous subsurface cold soils from a PHC-contaminated site located in Saskatoon Saskatchewan that are generally very low in organic matter with relatively high pH and Ca concentrations. This research will focus on the characterizing the role of absorption on P bioavailability in soils typical of our research partners sites in Saskatchewan that are affected by PHC contamination.

The general approach of this thesis is to utilize a combination of macroscopic experiments such as adsorption isotherms and adsorption/desorption kinetics studies with solid-state synchrotron spectroscopy to elucidate mechanisms of P retention and release. Collection of data from mineralogical and chemical techniques will allow the insight into the mechanisms of P transport and how they may be influenced by citrate addition and initial soil calcium levels.

Accordingly, the purpose of this study is to assess the chemical controls (Ca modification) on P transport through the soil and to determine the effects of soil properties (Ca, Mg, bulk density, pH, TIC, and TOC) on P transport through the soil while also considering the effectiveness of citrate as remediation agent. Additionally, the role of initial soil properties on P transport is crucial to future remediation. The thesis is organized as follows: Chapter 2 contains a brief literature review, followed by a characterization of soil cores from several site locations to produce a composite soil sample. Next, Chapter 3 covers steady-state studies of the extent of P sorption on the soils and mechanistic studies of P sorption using XANES spectroscopy. Then Chapter 4 a miscible displacement kinetics experiment was devised to simulate P adsorption and desorption in the presence of citrate under flow-through conditions typical of preferential flow paths. Finally, Chapter 5 summarizes the results so that they can be useful to both soil scientists and industrial research partners.

Figure 1.1: Photo of site in Saskatoon, SK. Previous bulk fuel station. Blue square illustrates exact location of soil collection.

Objectives

1. Determine the phosphate to citrate ratio needed to achieve 50% phosphate adsorption.
2. Determine the phosphate adsorption capacity of site soils (control and pretreated) using the reaction conditions expected onsite.
3. Determine the mechanisms of phosphate sorption and citrate interactions with phosphate on control and pre-treated soils, by understanding the fundamental mechanisms of Ca-citrate-P interactions using equilibrium and kinetics modeling in conjunction with XAS.

2. LITERATURE REVIEW

The purpose of this literature review is to provide a brief introduction and background for each research chapter below. This is to address any general information in common to both chapters or general background information that was not included in the two research chapters due to space/word limitations of peer-reviewed publications.

2.1 Hydrocarbon Soil Contamination

2.1.1 Contamination

Due to a combination of carcinogenic properties and high risk of transport, petroleum hydrocarbon (PHC) contaminated soils have strict regulatory requirements for remediation (Gao et al., 2015). Biodegradation of petroleum hydrocarbons is reduced in cold region soils, and follows a classical Arrhenius relationship of decreasing when the temperature decreases (Rike et al., 2003). In subsurface systems that have been contaminated with PHC, microbial degradation is often limited by nutrient availability, specifically phosphorus (P) (Leahy and Colwell, 1990). Thus, providing an effective source of nutrients to PHC-contaminated soils could greatly increase the rate of in-situ bioremediation and save both time and money to industry. The transport of P to the contaminant plume to stimulate remediation is important, but minimizing P mobilization offsite is equally important since P is of environmental concern in fresh water sources such as lakes, rivers and groundwater (Hsieh et al., 2007).

2.1.2 In-situ Remediation

In-situ remediation, in which microorganisms degrade PHC in the soil directly at the source, is more cost effective then standard ex-situ remediation, especially in urban areas (Das and Chandran, 2011; Siciliano et al., 2016; Bulmer et al., 2017). Microorganisms already present in ow temperature environments with contamination present are typically cold adapted for reproduction and degradation efficiency (Rike et al., 2003).

2.2 Study Site Characteristics

2.2.1 Soil characteristics

The system soils are calcareous subsurface cold soils. These soils are clay rich, very low in organic matter, and have high pH and calcium concentrations. Because of these soil chemical

properties, combined with near freezing temperatures for half of the year, and soil water moving through preferential flow paths, natural attenuation can be limited in these sites (Siciliano et al., 2016).

2.2.2 Parent Material

The parent material for the site is glacial till, which is typical for soils of the Canadian prairies. Although soils of the study site can be classified as heavy clay, mobilization of PHC and delivery amounts of amendment solution suggest that there are large preferential flow paths on the site. This leads to an implicit hypothesis that most of the chemistry takes place on the surface of the preferential flow paths, similar to what was observed by Gätcher, (Gätcher et al., 1998) in a loamy dystric Eutrochrept soil with a glacial till parent material located in the Central Swiss Plateau, Switzerland. Parent material is nonetheless useful as it is a starting point for the minerals present in which chemical sorption reactions are likely to occur. With Saskatchewan parent material being glacial till, carbonate minerals will play a major role in determining the effect that Ca^{2+} and Mg^{2+} have on P mobility, as both these ions react with phosphate anions (Siciliano et al., 2016; Bulmer et al., 2017; Hamilton et al., 2018). Understanding the concentrations of parent material base information allows for an estimation of required LMWOA to be applied to overcome the buffering capacity of the soil (Bulmer et al., 2017).

2.3 Chemistry of Biostimulatory Amendment Compounds

2.3.1 Phosphate

Phosphate (PO_4) chemistry in western Canadian soils is dominated by sorption reactions with calcium carbonate minerals and precipitation of calcium phosphate phases (Kolahchi and Jalali, 2013). These calcium phosphate phases limit the availability of amendment P to microbes, and therefore restrict transport of this essential nutrients to contamination of plumes in calcareous subsoils (Mills and Frankenberger, 1994). PO_4^{3-} is the most common form of phosphate in natural systems, this form of P is highly bioavailable when in a solution (Siciliano et al., 2016). The soil system has a strong affinity for P complexation through sorption processes, both through mineral complexation and precipitation of P-X minerals, mostly Ca-P mineral precipitates, these processes are primarily governed by pH (Tunesi et al., 1999; Manimel Wadu et al., 2013; Hamilton et al., 2018). (Peak et al., 2012) has studied the extent of Ca-P mineralization, with depends strongly on

the crystal structure of the mineral phase, further increasing the argument for pH importance in these calcareous soils.

2.3.2 Electron Acceptors

Terminal electron acceptors are often required in amendment solutions to avoid stalling remediation due to dilution of electron acceptors; for example, this research project uses magnesium sulfate ($MgSO_4$) as a background electrolyte and a primary electron acceptor. The stimulatory solution also contains some nitrate (limited by water quality regulations), which is similar to other researchers that used nitrate as an electron acceptor (Leahy and Colwell, 1990).

2.3.3 Magnesium

Research has concluded that Mg is an integral part in P mobility in calcareous soils, where Mg is involved with the creation of more soluble P compounds that increase the mobility of P with the addition of P in the form of potassium phosphate to a calcareous soil system, with some research suggesting that K_2HPO_4 may increase P solubility in combination with Mg/Ca (Manimel Wadu et al., 2013).

2.3.4 Low Molecular Weight Organic Acids

Low molecular weight organic acids (LMWOAs) can effectively solubilize P after its addition to the subsurface. The effects of LMWOAs have been studied on other soils, with some contradictory results. LMWOA addition has been shown to enhance P mobility, however sometimes mobility is decreased (Bolan and Naidu, 1994; Gao et al., 2010, 2015). LMWOAs occur widely and in multiple forms in natural soil environment as a result of plant and microbial exudation (Gao et al., 2015). Citrate is an LMWOA that is released by plant roots and is effective at increasing P bioavailability in soils (Duputel et al., 2013a). It is believed that LMWOA may also alter the availability of PAHs in the soil (Gao et al., 2010), as well as alter the amount of P sorbed onto the soil surface (Bolan and Naidu, 1994). Organic acids, particularly citrate (Oburger et al., 2011), extract greater amounts of P from soils that have been amended with phosphate fertilizers (Bolan and Naidu, 1994). However, other studies using a wide variety of soils observed that citrate can have an opposite effect on soils high in Ca; citrate addition can actually lower the amount of available P in Ca-rich soils containing smectites (Duputel et al., 2013a).

2.3.5 Ca Dissolution and Competition (Ca Modification)

In calcareous soils, the minerology of carbonate has an integral role in phosphate transport. The potential and likelihood of adsorption of phosphate onto these calcium minerals is high, which intern limit the mobility of P amendment (Siciliano et al., 2016). Researchers have discovered that calcite serves as a sink of phosphorus thus influencing its mobility (Xu et al., 2014). There is a high potential for calcite sorption of P in this soil; thus the formation of calcium phosphates onto carbonate minerals can reduce the available fraction of soil phosphate in a soil system (Siciliano et al., 2016). Calcium chemistry is important in our soil system as it is a place for P immobilization in the form of mineralization and precipitation of Ca-P minerals (Siciliano et al., 2016). Research in agricultural systems also illustrates the immobilization of P through adsorption of negatively charged phosphorus compounds onto minerals such as Al oxides or hydrous Fe clay minerals in the presence of polyvalent bridging cations like Ca^{2+} (Turner et al., 2005). This has been corroborated by research in other calcium-rich systems where reactions with calcium carbonates were rapid and formed a monolayer sorption onto $CaCO_3$ surfaces, resulting in dicalcium phosphates (Hussain et al., 2003).

2.4 Adsorption Processes in Soils

2.4.1 P Adsorption

A major limiting element in microbial growth is P, as microbes require P in biosynthesis of nucleic acids, fatty acids, ATP, and other cellular components. In many in-situ remediation practices, C:P ratios are maintained to boost bacterial biomass (Mills and Frankenberger, 1994). Phosphate in solution is a highly bioavailable form of P. In solution, P has tendency to adsorb to Fe, Al, and strongly with Ca minerals found largely in site soils (Siciliano et al., 2016). However, P in the soil solution has a high affinity for adsorption on the surface of iron oxide, aluminum oxide, and carbonate minerals. Only a small fraction of the total soil P is ever dissolved in solution; the dominant fraction is adsorbed. Laboratory-based adsorption studies are therefore commonly performed to quantify the capacity of a soil to retain/release P; the most common type of adsorption experiment is the adsorption isotherm.

2.4.2 Adsorption Isotherms

The maximum adsorption capacity of a chemical is an important measurement for not only fundamental soil chemical experiments but also in geochemical transport modeling and risk

assessment. Experimentally, adsorption isotherms (varying the amount of reactant with all other parameters held constant) and the models that describe the resulting sorption (partitioning constants, Langmuir isotherms, etc.) can provide a suitable data set for the characterization of sorbate adsorption onto sorbents (Hsieh et al., 2007; Oburger et al., 2011; Plazinski et al., 2013). With the help of isotherm models, parameters such as the maximum value of P adsorption and the affinity of P for a soil can be determined. The parameters varied in a traditional batch adsorption isotherm experiment is the initial reaction concentration; fixed parameters include solid density, ionic strength, pH, and temperature. In general adsorption isotherms the solutions are allowed to equilibrate (24-48hrs), the solution is then centrifuged and the final concentration is then measured. By difference, a mass adsorbed can be determined. This is illustrated in Figure 2.1. This is a common practice for determining the adsorption capacity of a substance (Smith et al., 2003; Kao et al., 2003; Riahi et al., 2013; Chen, 2015).

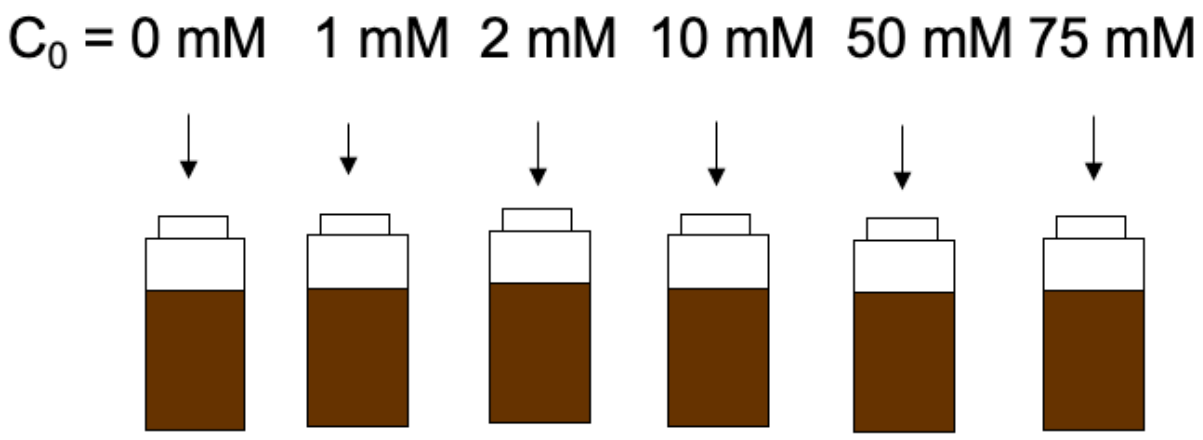

Figure 2.1: Conceptual model of batch adsorption isotherms. Where a known concentration of citrate was added to the soil solution.

The following is an example calculation on determining the amount of P sorbed onto the soil.

C_0-C_f = C_{sorbed}

V_0C_0-V_fC_f/mass sorbent = q (amount sorbed per unit mass soil)

q vs. C_f = adsorption isotherm

Once experimental data is collected then it can be modelled using a variety of conceptual models, including Langmuir, Freundlich, and linear models.

2.4.3 Langmuir Isotherm Modeling.

The Langmuir model (equation located in Table 2.1, curve described in Figure 2.2) is a well-known nonlinear adsorption model that assumes that monolayer coverage occurs at equilibrium, and all adsorption sites are energetically equivalent. Qualitatively, it is characterized by an adsorption plateau where saturation occurs such that once a molecule occupies a site, no further adsorption can take place. Moreover, Langmuir theory has related rapid decrease of the intermolecular attractive forces to the rise of distance (Bolster and Hornberger, 2008). This model has been successfully used in many adsorption studies (Karadag et al., 2007; Bolster and Hornberger, 2008; Penn, 2011; Stoner et al., 2011; Chen, 2015). Parameters in the Langmuir model are as follows:

C_e = denote equilibrium concentrations of adsorbate (mg L^{-1})

q_e = the adsorption capacity at equilibrium (mg g^{-1})

q_o = is the maximum amount adsorbed required to saturate a unit mass of adsorbent (mg g^{-1})

K_L = Langmuir constant representing free energy of ion-exchange (energy of adsorption) (mg L^{-1})

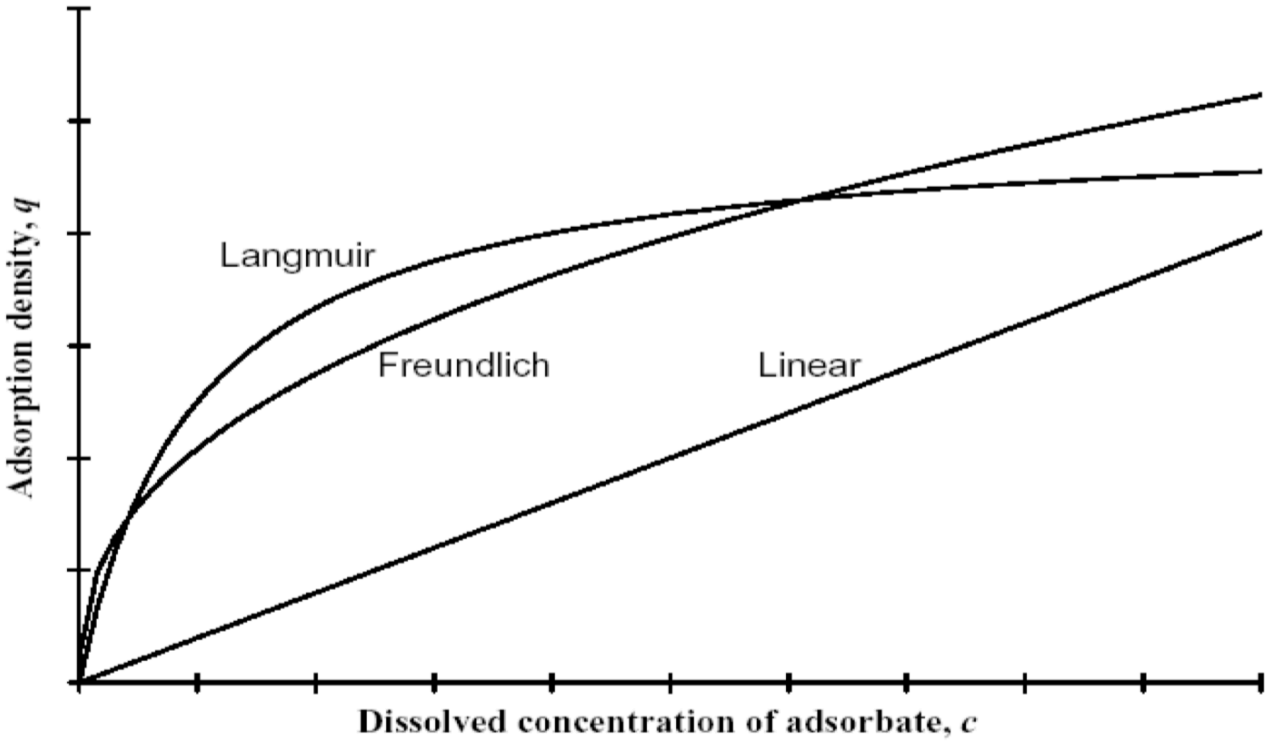

Figure 2.2: Conceptualization of Linear, Langmuir, and Freundlich models.

2.4.3 Freundlich isotherm modeling.

The Freundlich equation (Freundlich., 1907) is also located in table 2.1 and described in Figure 2.2. Originally developed to determine the adsorption of gas onto solids, it has been found to often describe sorption reactions in soils for a variety of adsorption systems. The variables related to the Freundlich isotherm in table 2.1 are defined as follows:

C_e = denote equilibrium concentrations of solution (mg L^{-1})

q_e = the adsorption capacity at equilibrium (mg g^{-1})

1/n = values indicate the type of isotherm to be irreversible (1/n = 0), favorable (0 < 1/n< 1), and unfavorable (1/n>1).

K_f = is the Freundlich adsorption capacity at unit concentration (energy of adsorption) (mg L^{-1}); the higher the K_f the higher the maximum sorption capacity. The 1/n, term relates to the intensity of adsorption; with increased 1/n value adsorption becomes more likely to occur. This model is

considered a multi-layer processes, in which the amount of adsorbed solute per unit adsorbent mass increases gradually (Chung et al., 2015).

Table 2.1: Tabulated information of Langmuir and Freundlich models.

Kinetic Model	Linearized Form	Plots	Slopes	Intercepts
Langmuir	$C_e/q_e = 1/q_oK_L+C_e/q_o$	C_e/q_e vs C_e	1/Qm	1/QmKl
Freundlich	$\ln(q_e) = \ln Kf +(1/n)\ln(C_e)$	$\ln(q_e)$ vs $\ln(C_e)$	1/n	Log Kf

2.5 Adsorption Kinetics

Sorption processes may not reach equilibrium in preferential flow conditions (Smith et al., 2003), therefore sorption kinetics are useful to estimate the rates of P sorption and release on the soil. P adsorption occurs through a biphasic mechanism that has a fast adsorption rate followed by a slower desorption rate (Peak et al., 2012). Following the initial adsorption, a slower continued sorption of P has been ascribed to diffusion into micropores or aggregates, which is slower than adsorption (Kolahchi and Jalali, 2013). Kinetics of P sorption is important to P leaching because the soil may have a limited amount of time to be in contact with site specific soil solution, therefore the rate of reaction is important (Penn et al., 2013). A variety of methods (stirred flow, miscible displacement, and spectroscopic techniques) have been employed in past research to help determine phosphorus chemistry on soil surfaces (Smith et al., 2003; Peak et al., 2012; Penn et al., 2013).

The importance of desorption is often overlooked in kinetics experiments, with preferential treatment given to the rates of adsorption. However, the rates at which a substance is removed from a sorbent is of vital importance to soil chemists, as it provides insight into how strongly it is held to a surface or into the sorbent matrix. For instance, diffusion-driven reactions result in molecules inside the soil matrix that can be less available for release vs molecules adsorbed onto the surface of a particular sorbent (Smith et al., 2003).

2.5.1. Miscible Displacement Kinetics Experiments.

One common method for conducting adsorption kinetics experiments is the use of a continuous flow miscible displacement (thin disk) experimental design. An experimental example is located below in Figure 2.5. Where you can see the trend of adsorption onto an experimental substance and once reached equilibrium the molecules to be adsorbed are switched off resulting in a sharp decrease of concentration. A further illustration is illustrated in figure 2.3, where initially the flow of solution is pumped through an apparatus containing the sorbent, in this case soil. Where a fraction collector retrieves a sample at a predetermined time. This is widely used experimental design for studying reactions that have durations of minutes to days (Smith et al., 2003; Kolahchi and Jalali, 2013; Selim et al., 2013).

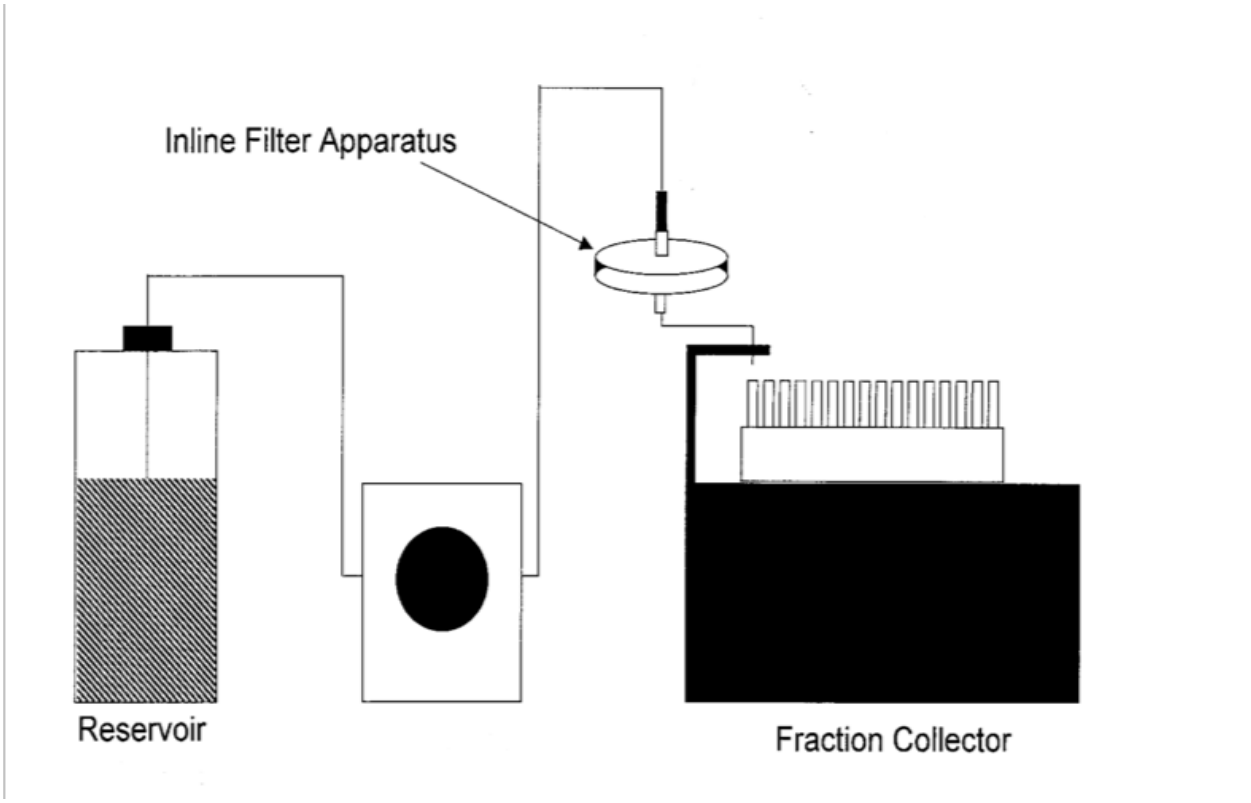

Figure 2.3: Conceptual model of sorption kinetics apparatus on a soil. Fluid flows via peristaltic pump into a inline filter apparatus and collected at a predetermined interval adapted from (Smith et al., 2003)

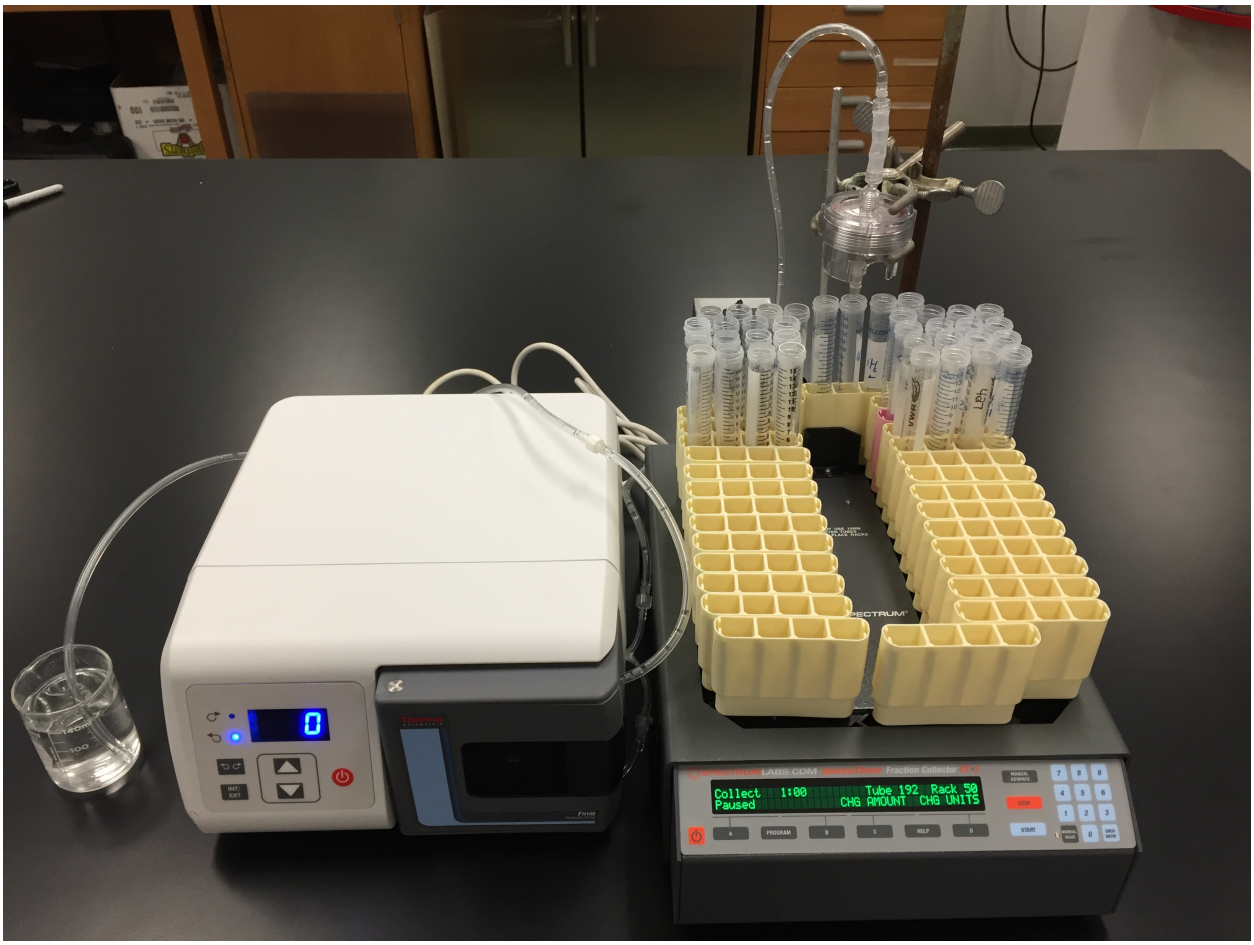

Figure 2.4: Kinetics experimental set up (actual), with fraction collector(right), peristaltic pump (left).

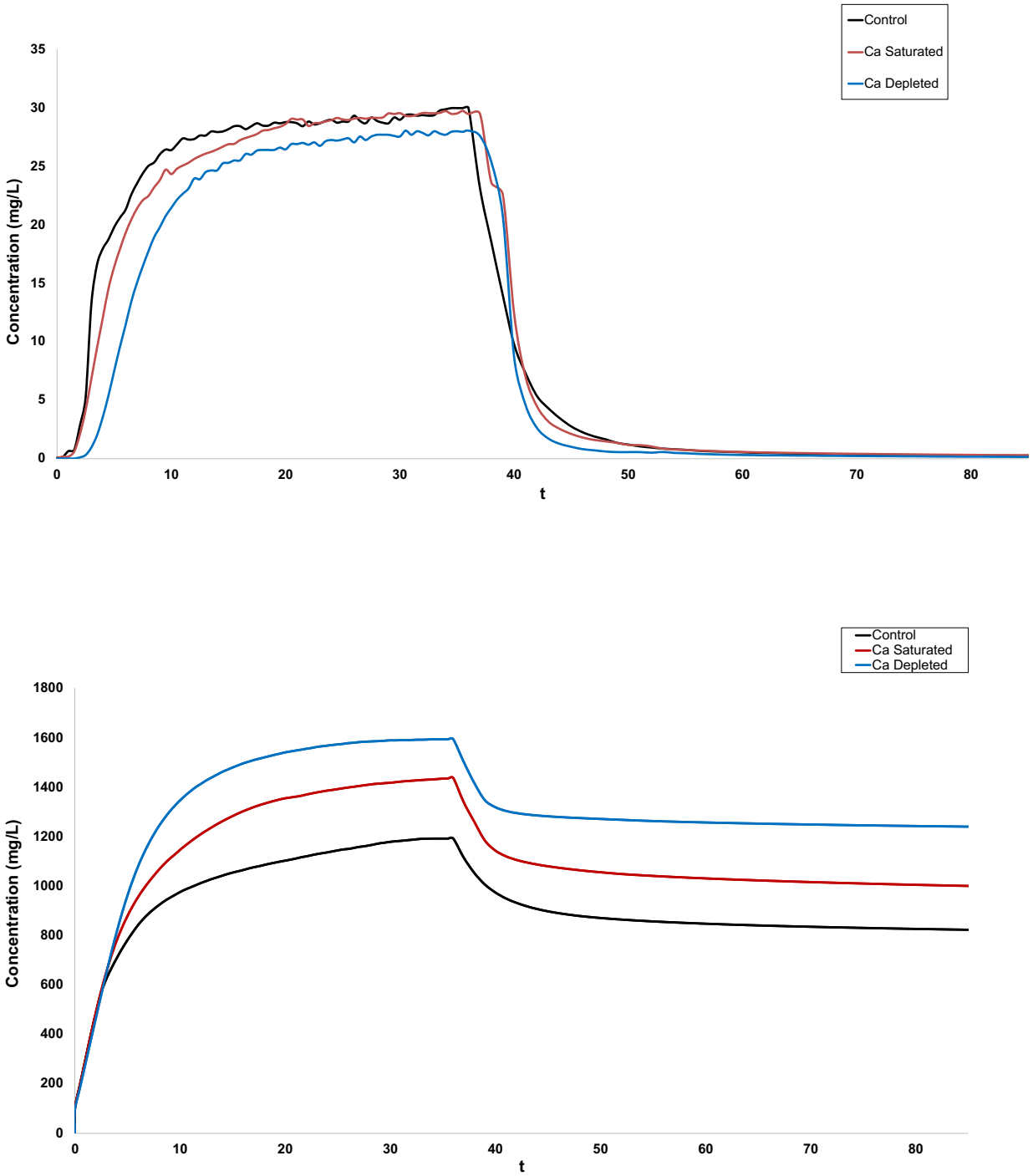

Figure 2.5: Experimental data from a typical miscible displacement (thin disk) experiment reported as mg L^{-1} P (on top) and converted to q (mg P sorbed/kg soil) (bottom). These curves are typical for miscible displacements experiments.

2.5.2. Kinetic Models.

The following describes the most common kinetic models applicable to the data retrieved from a miscible displacement experiment; equations are tabulated in Table 2.2 and a discussion of the model variables follows. Some models are based upon reaction-rate limited (chemical kinetics) systems, and some are based upon diffusion-limited (mass transfer based) systems. Conceptually, you can visualize the observed kinetic rates as being limited by the slowest step in the process, which are illustrated in Figure 2.6.

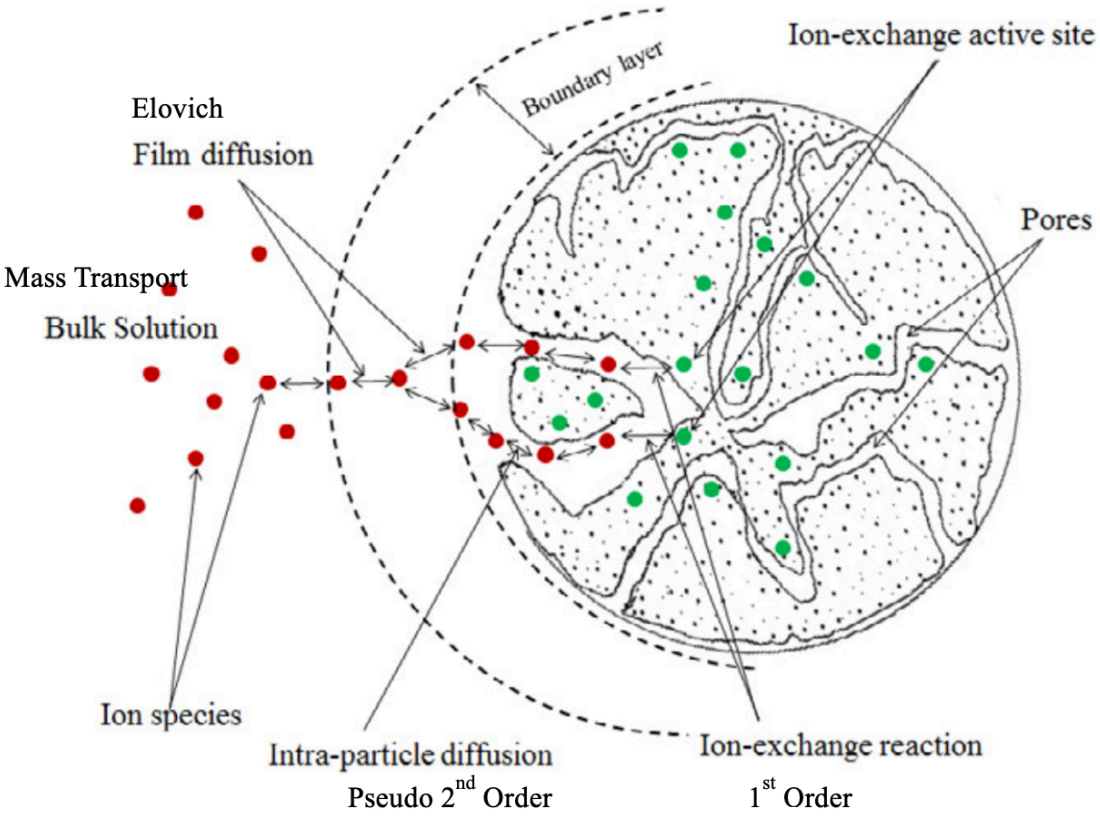

Figure 2.6: Conceptual model of sorption kinetics on a colloid that depicts possible sorption kinetic models being rate limiting; for equations see table 2.1 and 2.2. Adapted from (Al-Jubouri and Holmes, 2017).

Table 2.2: Compilation of kinetics models and equations.

Kinetic Model	Linearized Form	Plots	Slopes	Intercepts
Pseudo-first order	$\log(q_e-q_t) = \log(q_e) - k_i/2.303t$	$\log(q_e-q_t)$ vs t	$-k_i/2.303$	$\log(q_e)$
Pseudo-second order	$t/q_t = 1/k_2q^2_e + 1/q_e\ t$	t/q_t vs t	$1/q_e$	$1/k_2q^2_e$
Intra-particle diffusion	$q_t = k_dt^{1/2} + C$	q_t vs $t^{1/2}$	k_d	C
Elovich	$q_t = 1/\beta\ \ln(\alpha\beta) + 1/\beta\ \ln(t)$	qt vs ln(*t*)	$1/\beta$	$1/\beta\ \ln(\alpha\beta)$

2.5.2.1 Pseudo First Order Model.

The Pseudo-first and pseudo-second order kinetics models (Lagergren, 1898; Ho and Mckay, 1998; Mckay et al., 1999; Azizian, 2004; Riahi et al., 2013) are tabulated in table 2.1 in linearized form with plots, slopes, and intercepts derived via integration and applying the boundary conditions of the following t=0 to t=t and q_t=0 to q_t=q_t .

The values are defined as follows:

q_e = equilibrium sorption capacity (mgL^{-1})

q_t = sorption capacity at time t (mgL^{-1})

k_1 = rate constant of the pseudo-first order chemical sorption (min^{-1})

The premise of the pseudo first order kinetics model is that the concentration of one reactant remains constant because it is supplied in great excess; this reactant can be then be absorbed throughout the process without solution concentrations changing; this is the method of determining the rate constant of the pseudo first order reaction constant

2.5.2.2 Pseudo Second Order Model.

The pseudo-second order kinetics model (Ho and Mckay, 1998a; b, 1999; Vasanth Kumar et al., 2005; Ho, 2006; Plazinski et al., 2013), as illustrated in table 2.1 describes the pseudo-second order equation as being based on the sorption capacity on the solid phase.

The values are defined as follows:

q_e = equilibrium sorption capacity ($mg\ g^{-1}$)

q_t = sorption capacity at time t ($mg\ g^{-1}$)

k_2 = rate constant of the pseudo-second order sorption ($g\ mg^{-1}\ min^{-1}$)

This model predicts the behavior of the data over a much wider range of reaction times in soil chemical processes and assumes that chemisorption is the rate-controlling step. The definition of chemisorption by (Vallero, 2014) is the process of integrating a chemical into porous materials surface via chemical reaction. The pseudo second order equation has a significant advantage in

modeling. The model does not have issues determining an effective adsorption capacity, with the mechanisms of which being the rate constant, or initial adsorption rate (Ho, 2006). This model does not struggle with assigning a q_e value, thus making it a good choice for effective sorption kinetics in systems where isotherm parameters are also desired.

Use of the second order kinetics model has been used with success on a variety of different systems; strong correlation between the models theoretical results and experimental results have been reported in systems where chemisorption between sorbent and sorbate occurs (Ho and McKay, 1999). McKay concluded that for many chemically-rate limited systems, the pseudo second order kinetics model was preferred because other models, such as first order kinetics, only fit part of the data and not the whole set (Ho and McKay, 1999). The most commonly applied form of the pseudo second order equation (PSOE) applied to describe the solid/solute sorption was proposed by Ho and MaKay, 1998 (Plazinski et al., 2013). The PSOE has a high correlation to a very large spectrum of kinetic data, with many diverse systems being defined using this model, this indicates the model represents more than one kinetic model. This high correlation to many different systems is usually associated with the rate of direct adsorption/desorption processes on the surface of the sorbate which controls the overall sorption kinetics (Plazinski et al., 2013). The model has been applied extensively in other systems (Hu et al., 2016), which applied the model to correlate the experimental kinetic data with a chemical exchange, and invoked valence forces by sharing or electrons exchange between solute and the ion-exchange material, which in turn determined the rate of reaction, and found that Q_{max} of both the model and the experimental data was correlated with great success (Al-Jubouri and Holmes, 2017).

2.5.2.3 Intra-Particle Diffusion.

The intra-particle diffusion kinetics model originally by (Weber and Morris, 1963) as illustrated in table 2.2, describes the process of a sorbate diffusing and adsorption onto the porous particle surface (Plazinski and Rudzinski, 2009). This model's rate limiting step is a combination of film diffusion and intra-particle mechanisms (including diffusion through the porous materials) (Al-Jubouri and Holmes, 2017).

The values are defined as follows:

k_d = is the intra-particle diffusion rate constant (mg g^{-1} $min^{1/2}$).

q_t = sorption capacity at time t (mg g^{-1})

C = boundary layer thickness in the rate controlling step (mg g^{-1})

2.5.2.4 Elovich Equation.

The Elovich equation (Low, 1960) as described in table 2.2 relies upon integration of the rate equation with the same boundary conditions as the pseudo first and second-order equations but is valid for diffusion-limited reaction conditions. The values are defined as follows:

α = initial adsorption rate (mg g^{-1})

β = related to the extent of surface coverage and activation energy for chemisorption (mg g^{-1})

q_t = is the quantity of gas adsorbed during time t (mg g^{-1})

The Elovich equation is generally used to determine the kinetics of chemisorption of gases onto a heterogeneous solid substance with a very restrictive nature (Ho, 2006; Ncibi et al., 2008; Betts et al., 2013; Riahi et al., 2013). The Elovich equation's rate limiting step is generally considered film diffusion, thus it can be concluded that diffusion is the primary mechanism of action, in this case, this equation should be used when desorption rates can be neglected (Chakrapani et al., 2010).

2.6 Rationale for Thesis Research

The transport of nutrients to the contaminated plume to stimulate remediation is important, but minimizing nutrient mobilization off-site may be equally important as P is an element of concern in lakes, rivers and groundwater (Hsieh et al., 2007). For these reasons, not only short-term concentrations but also longer-term chemical fate is important for biostimulatory amendment research. The use of LMWOA are not well understood in remedial systems. By developing a deeper understanding of citrate, Ca, and P interactions on calcareous soils and which concentrations these elements play a competing role, we can contribute to the overall understanding of in situ remediation process design. More broadly, understanding the relationships between soil parent materials and transport of ions through the soil system is of general interest to soil chemists. Specifically, how Ca and citric acid interact with PO_4 in a soil is required to determine the optimal amount of citric acid to add to contaminated sites to allow P to be desorbed and transported to the contaminated plume. The rate at which this happens allows for a "ball park" timeline for PHC biostimulation to occur.

3. EXTENT AND MECHANISM OF INTERACTION BETWEEN PHOSPHATE AND CITRATE IN A CALCAREOUS SOIL

3.1 Preface

The effects of different rates of citrate and soil calcium concentration on soil in-situ bio remediation dynamics is important to determine before large scale industrial practices can be implemented for the sustainable remediation of hydrocarbon contaminated sites in Saskatchewan. A long-term research trial at the Broadway Saskatoon site in partnership with Federated Co-op was used as the source material for this lab scale principles paper. This was used to compare the difference in citrate and calcium concentrations and what amount of citrate was required to get site soils to adsorb 50% of the P compared to the control. This chapter covers the influence of citrate and calcium on P mobility of our site soils, how the addition of citrate effects the mobility of P, and how calcium levels can interfere with P transport in the presence of citrate. This paper was published in SSSAJ December 2017.[1]

3.2 Abstract

In subsurface systems that have been contaminated with petroleum hydrocarbons (PHC), the degradation by microbes is limited by nutrient availability, primarily by bioavailable phosphorus (P). Low molecular weight organic acids (LMWOA), particularly citrate, have been proposed as a remediation amendment to enhance P mobility, but the effect of citrate on P retention by calcium-rich soils is poorly understood. In this study, the sorption of P in the absence and presence of citrate on calcareous cold soils was investigated under controlled laboratory conditions using a combination of lab-based adsorption and desorption studies and synchrotron-based spectroscopic techniques. The results indicate that calcium exerts a strong control on P sorption by forming calcium phosphate minerals, thus limiting the availability of P for transport or microbial uptake.

[1] Bulmer, D., Kar, G., Hamilton, J., Siciliano, S., & Peak, D. (2018). Extent and mechanism of interaction between phosphate and citrate in a calcareous soil. *Soil Science Society of America Journal, 82*(2), 315-322. 10.2136/sssaj2017.08.0289

Treatments that reduce, reserve, and exchangeable Ca increase the relative proportion of adsorbed P. When citric acid is also added to these calcareous soils, their buffering capacity largely inhibits any desorption of P or changes in P speciation until citrate levels exceed P additions by more than 10 to 1. By increasing citrate:P ratios to between 10:1 and 50:1, it is possible to overcome the soil's buffering capacity, decrease pH, and enhance P desorption via competition with citric acid. This suggests that one can enhance P transport in this soil system, but only when the citrate to P ratio is properly adjusted for the site's Ca levels.

3.3 Introduction

In petroleum hydrocarbon contaminated (PHC) subsurface systems, microbial degradation of PHC is often limited by nutrient availability, specifically phosphorus (P) (Leahy and Colwell, 1990; Duputel et al., 2013a). By providing an effective source of nutrients to PHC - contaminated soils, in-situ biostimulation can be achieved. The transport of nutrients to the contaminant plume to stimulate remediation is key, but also minimizing nutrient mobilization offsite is important since P is an element of concern in lakes, rivers, and groundwater (Hsieh et al., 2007). The most common species of P in natural environments is phosphate (PO_4), and the most bioaccessible and mobile form of PO_4 is dissolved in solution (Siciliano et al., 2016). However, sorption processes strongly bind PO_4 to mineral fraction of soils. Specifically, calcareous subsurface cold soils that are high in clay and carbonate minerals readily form calcium phosphate (Ca-P) minerals that limit both bioaccessibility and transport of PO_4 (Hannapel et al., 1964; Wandruszka, 2006; Kolahchi and Jalali, 2013).

Carbonate minerals are also an important adsorbent in these calcareous soils (Wandruszka, 2006; Xu et al., 2014; Siciliano et al., 2016). These soils are high in both Ca^{2+} and Mg^{2+}, and both ions react with phosphate anions. With increased levels of carbonates allow for increased buffer changes in pH due to addition of low molecular weight organic acids (LMWOA) such as citric acid. With the addition of acid, carbonate minerals dissolve and release Ca and form sparingly soluble Ca-P phases, thus precipitating P from solution (Tunesi et al., 1999; Cao and Harris, 2008; Stoner et al., 2011). The formation of calcium phosphate minerals typically limits solubility of P in neutral and alkaline soils, with pH affecting the stable Ca-P phase that forms thus reducing the availability of P. At 1mM this was the maximum P that can be added without exceeding saturation indices for calcium phosphate minerals at pH 6.5 At pH 6-7, more brushite ($CaH_2PO_4*2H_2O$) typically forms whereas increased pH results in more hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) formation

in soil (Brookins, 1988; Peak et al., 2012). Generally, increases in soil pH causes calcium phosphate solubility to decrease (Baker et al., 1998), and increases in Ca:P ratio also decrease solubility via shifting equilibria from monocalcium phosphate minerals such as brushite to polycalcium minerals such as hydroxyapatite (Dorozhkin, 2011).

In a recent study (Siciliano et al., 2016), the relationship between phosphate speciation and bioremediation using large bore injectors to provide continuous low-rate phosphate amendment to the site was examined. We learned that adsorbed phosphate was bioaccessible to microbes degrading PHC in a calcareous soil and that changing the soil Ca and Mg concentrations had a significant effect on speciation of P, microbial community composition, and microbial degradation activity. Finally, we learned that some phosphate minerals such as newberyite, $MgHPO_4 2H_2O$ and brushite, $CaHPO_4 2H_2O$, significantly decreased degradation, suggesting that enhancing solubility and increasing the relative amounts of adsorbed P is key for biostimulation. This led to a hypothesis that an LMWOA such as citrate could serve a biostimulatory amendment in this site.

Phosphate and LMWOAs both also have a high adsorption affinity onto metal oxides, such as iron and aluminum oxides, and a high affinity to form complexes with dissolved Ca (Gätcher et al., 1998; Oburger et al., 2011). Thus, LMWOAs can compete with P for the same adsorption sites via ligand exchange and/or decrease sorption sites in soils via ligand-promoted dissolution of minerals (Strawn and Sparks, 2000; Oburger et al., 2011; Stoner et al., 2011). However, there are conflicting results in the literature about the effectiveness of LMWOAs. One of the most effective LMWOAs to solubilize P is citric acid (Geelhoed et al., 1998; Duputel et al., 2013a; Siciliano et al., 2016), but sometimes additions of citric acid enhanced P mobility (by effectively preventing adsorption or precipitation) whereas sometimes the mobility of P is decreased (Bolan and Naidu, 1994; Gao et al., 2010; Duputel et al., 2013b; Siciliano et al., 2016). A recent study suggested that the decrease in P mobility with the addition of citric acid is due to adsorption of the citrate anion resulting in more negative surface charge of clay mineral sorbents, which increases adsorption of Ca^{2+} ions onto clay minerals through electrostatic interactions. Such adsorption of additional Ca^{2+} creates positive surface functional groups for PO_4 adsorption (Duputel et al., 2013a). In addition to its chemical properties, citric acid is non-toxic and available in industrial quantities, making it an attractive candidate for addition as a biostimulatory amendment. Our previous research in P bioavailability and speciation in a similar field site (Siciliano et al., 2016) led us to conclude that soils of this region might respond well to citrate additions.

Accordingly, this study was designed to test the relative importance of varying Ca and citric acid upon PO_4 sorption in a calcareous soil from an active bioremediation site. We hypothesized that the addition of citric acid to the soil system would decrease the amount of P amendment solution adsorbed onto soil particles, and the amount of Ca in the soil would result in different mechanism of interactions between P and citrate. To determine the influence of Ca upon P sorption, a pre-treatment to either remove soil calcium or provide excess soil Ca without substantially changing the soil mineralogy was used to produce three treatments: Control, Ca saturated, and Ca depleted (Wandruszka, 2006; Manimel Wadu et al., 2013). Phosphate sorption in the presence of a range of citric acid concentrations on these three Ca treatments was then characterized via colorimetric and synchrotron-based approaches to elucidate the effect that citric acid and calcium have upon phosphate binding and to identify the optimal concentration of citric acid to include in a biostimulatory solution for an active research field site undergoing bioremediation with amendments of PO_4 (Siciliano et al., 2016).

3.4 Materials and Methods

All chemicals used in this study were ACS reagent grade or better and purchased from Fisher Scientific.

3.4.1 Soil Samples and Pretreatments. The soil samples for laboratory experiments were collected as outlined by Siciliano et al., 2016, using a push core drill to collect samples at two different sites (Broadway South Side, and Broadway West Side) in Saskatoon, Saskatchewan, Canada. A total of ten different cores (including different depths) were collected. Soils were hydrocarbon contaminated for several years and frozen for 4-6 months a year. After soils were collected, they were stored at -20°C. After initial screening to verify that the texture (Djodjic, 2004; Zheng and Zhang, 2011), and basic properties of the individual samples were similar, all subsamples of the cores were consolidated to produce a single bulk sample representative of the site. This was done out of necessity to produce enough soil for this study and gives a better site representative soil. The consolidated soil is a clay loam (27% sand, 41% silt, and 32% clay) with bulk density of 1.3 g cm^{-3}.

Two experimental manipulations of the consolidated soil (which hence is referred to as control) were conducted to produce a calcium-depleted and a calcium-saturated soil. The method for soil treatment is outlined by Siciliano et al., 2016. Briefly, the control soil was not treated, the Ca-depleted soil was prepared by adding deionized water to a 1:1 soil slurry, and further reducing

calcium concentrations in the soil through a 1.0 M $MgCl_2$ solution while also adjusting pH to 6.5 with 1.0 M H_2SO_4, which was then centrifuged and washed three times, therefore removing excess ions as well as $CaSO_{4(s)}$. The soil was then added to a 1.0 M $MgCl_2$ solution in a 1:4 ratio, allowed to react overnight with mild stirring, then washed three times with deionized water. This entire process was repeated three times to exchange bound (adsorbed) Ca^{2+} with Mg^{2+}. The Ca-Saturated soil was made through the addition of 1 M $CaCl_2$ in a 1:4 ratio and stirred with a magnetic stir overnight, then washed via centrifugation three times with double deionized water (DDI) to remove excess ions. This process was repeated three times to increase the amount of bound Ca^{2+} in the soil. All soils including control were oven dried at 80°C to remove any excess moisture before experimental use.

3.4.2 Total Elemental Analysis. Soil organic carbon (as percent) was determined via loss of mass in H_2O_2 oxidation during particle size analysis. Other elements were analyzed via X-ray fluorescence (XRF) using a Thermo Fisher Scientific ARL OPTIM'X X-ray Analyzer. Air-dried soil samples were ground by hand with an agate mortar and pestle and then analyzed as powders under helium X-ray chamber conditions. Elemental counts per second were converted to mg kg^{-1} concentrations using the Thermo Fisher Scientific OPTIQUANT software package. This software package provides an accuracy to within 10% of each element. A known standard reference soil (NIST 2711) was periodically measured as a quality check.

3.4.3 Soil Surface Area. A consolidated sample from each soil treatment was analyzed for external surface area via 3 point BET (Brunauer-Emmett-Teller) surface area analyzer using an ASAP 2020 by Micromeritics..

3.4.4 X-ray diffraction. Synchrotron powder diffraction measurements to determine mineralogical changes upon pre-treatment were collected at the Canadian Light Source (CLS) CMCF-BM beamline (08B1-1) using a wide-area detector. The data was processed using GSAS-II (Toby and Von Dreele, 2013) with phase identification performed using X'pert Highscore Plus software v. 3.0 (PANalytical, Inc.).

3.4.5 Adsorption Isotherms. P adsorption experiments were conducted in quadruplicate to determine steady state P adsorption affinity at a pH of 6.5. This pH was chosen because it corresponds to the pH of the amendment solution added to the field site (Siciliano et al., 2016). As the site naturally is high in both primary and secondary carbonate minerals, it buffers the addition of a slightly acidic solution. Air dried soil (1.0g) was weighed out into a 50ml falcon tube. A stock P solution from KH_2PO_4 was added in the following concentrations, 0, 2, 4, 8, 10, 20, 40,

60, 80, 100 mg L^{-1} P. A background electrolyte of 0.011M $MgSO_4$ adjusted to pH 6.5 as used in (Siciliano et al., 2016) was applied in all cases. All samples were shaken for 24 hours and filtered at 0.45µm before measurement. The final measure was taken on the Seal Analytical Auto Analyzer (Seal Analytical, Germany), through the colorimetrical analysis using a molybdenum blue method (Murphy J., 1962). The adsorption isotherms were modeled to the Langmuir equation as it provided the most strongly correlated model. The citric acid isotherms followed the same basic isotherm principal explained above, with a minor change. A predetermined concentration of P (1 mM) was kept constant while different concentrations of citric acid were added at each of the following concentrations: 0, 0.5, 1, 2, 3, 4, 5, 10, 25, and 50 mM. A nitric acid equal molar concentration was added to determine the effect of soil buffering capacity.

3.4.6 P K-edge XANES spectroscopy. P K-edge X-ray absorption near edge structure (XANES) spectra were collected on the Soft X-Ray Microcharacterization Beamline (06-B1-1) of the Canadian Light Source in partial fluorescence mode, using 4 element solid state detector (Bruker). Data was processed and analyzed using the Athena software package (Ravel and Newville, 2005). Linear combination fitting (LCF) was performed on adsorption samples using a large library of high quality phosphate reference compounds that included adsorbed phosphate, phosphate minerals with a range of cations, and organic phosphates (Kar et al., 2017). Standards were diluted in boron nitride to avoid saturation effects on XANES from self-absorption. The principles used to develop the LCF model was based upon (a) using the minimum number of standards for the dataset to reproduce the samples with acceptable goodness of fit (quantified via Athena's reduced chi squared). (b) Preference was given to phosphate minerals that were stable at the pH of the soils (brushite, newberyite at pH 6) over other minerals when similar fits were achieved.

3.4.7 Statistical Analysis. All adsorption data on the effect of treatments and citrate were subjected to analysis of variance (ANOVA) using PROC MIXED in SAS (Statistical Analysis System, Version 9.4 for Windows™; SAS Institute, Cary, NC). The denominator degrees of freedom method (*ddfm*) option will use the Satterthwaite method. Multi-treatment comparisons of variables are made using the Tukey's studentized range test method of mean separation at the probability level of $P \leq 0.05$ to establish statistical significance, where pdmix800 SAS macro (Saxton, 1998) was used to assign grouping.

3.5 Results

The calcium content of the soil was modified by the treatments, with a large decrease in the Ca-depleted soil compared to the control and a small increase in the saturated soil both suggesting that the treatments were effective in adjusting Ca content (Table 3.1). Furthermore, XRD (in Figure 3a) revealed changes in calcite among the treatments, with more amorphous material forming in the Ca-saturated soil (appearance of a broad baseline), and a reduced calcite presence in the Ca-depleted soil relative to the control. Interestingly, there was some evidence from XRD that the pre-treatments shifted smectite in the controls to more vermiculitic (Ca saturated) or chloritic (Ca depleted) phases. Magnesium concentrations also increased in the depleted treatment (Table 3.1) which was expected as a side effect of the treatment.

Table 3.1: Basic soil properties and PO_4 adsorption parameters for the three treatments.

Treatment	pH‡	Texture	TIC¶	Total Elemental Concentrations†§						Langmuir Parameters		
				Fe	P	Ca	Mg	Ca/P	Ca/Mg	Smax	K	R^2
			%	mg kg^{-1}						mg kg^{-1}	1 kg^{-1}	
Control	7.0	Clay Loam	1.02	28100 (1967)	994 (9.9)	15800 (790)	6810 (204.3)	15.90	2.32	379.2	0.0025	0.99
Depleted	7.4	Clay Loam	0.77	24400 (1708)	959 (9.6)	5270 (158.1)	10400 (416)	5.50	0.51	531.6	0.0017	0.98
Saturated	7.2	Clay Loam	0.69	29300 (2051)	1000 (10)	18300 (1098)	6460 (193.8)	18.3	2.83	674.9	0.0014	0.98

† Total elemental compositions were obtained through X-ray fluorescence.

‡ Soil samples were measured at pH 6.5, the native soil is pH ~7.

¶ TIC, total inorganic carbon. Organic carbon composition contained hydrocarbons and was initially high; these values are from soil that has had hydrocarbons volatilized.

§ Values in parentheses are standard deviation.

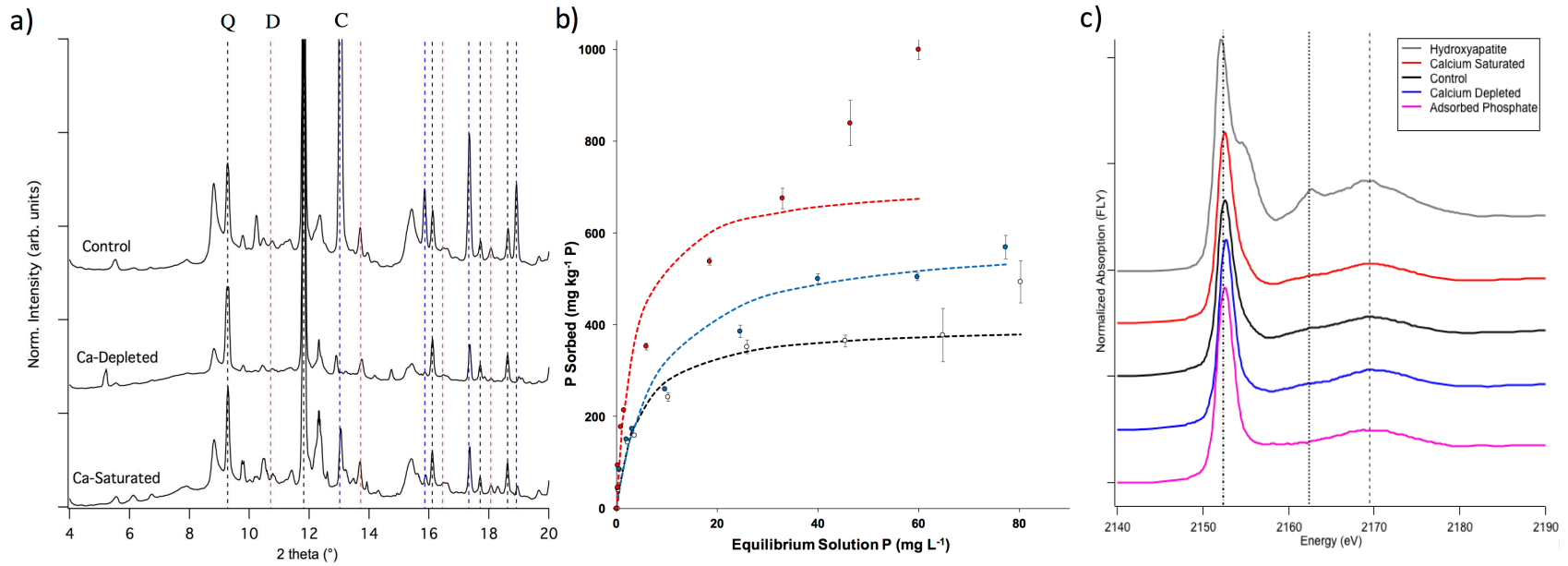

Fig. 3.1: (a) Synchrotron powder x-ray diffraction on two soil pretreatments, as well as the control soil from the Broadway site. Q, quartz; D, dolomite; C, calcite. (b) Phosphate adsorption isotherms on control soil (black circles), Ca-depleted soil (blue circles), and Ca-saturated soil (red circles) at pH 6.5. Dashed lines denote the fit of the Langmuir adsorption model to the data. (c) Phosphorus x-ray K-edge absorption near edge structure of sorption samples with a predetermined P concentration (1 mM) compared with P reference compounds.

Both treatments have more P adsorption than the untreated soil (control) (Figure 3.1b); this is likely due to interactions with Ca or Mg rather than changes in surface area/particle size since control, depleted, and saturated soils had BET value of 31.5, 23.4, and 19.1 m^2g^{-1}, respectively. It is possible that the decline in surface area upon treatment is due to conversion or loss of the smectite clays in the control; the loss in surface area did not negatively affect adsorption capacity implying that clay minerals are not the major sorbent for phosphate in these soils. From the sharp increase in adsorption that occurs above 31 mg L^{-1} P in the Ca-saturated experiments, it appears that precipitation with Ca occurs above this concentration. The overall model of best fit for the isotherms was the Langmuir model; this model was chosen because it was the model that could best describe all three treatments from 0 to 31 mgL^{-1}; results are tabulated in Table 3.1. The Langmuir model had an average R^2 of 0.983 compared to 0.960 for Freundlich (Freundlich model not shown). From the Langmuir parameters, one can see that the maximum adsorption changes with the modification of Ca levels in the soil, further significance is illustrated in figure 3.2. P K-edge XANES was performed on samples from each treatment after 24 hours of reaction with P in a background electrolyte of $MgSO_4$ as previously described. The results are shown in Figure 3.1c compared to standards of phosphate adsorbed on goethite and a crystalline hydroxylapatite. Based upon XANES, there is evidence of some Ca-P structure in all three soil samples (see dotted lines in Figure 3.1c), but no clear changes in chemical speciation as a function of treatment suggesting that sorption is similar in all cases in the absence of citrate.

Varying concentrations of citrate enhanced P desorption independent of changes in pH in our soil experiments. The effect of citrate upon P sorption was experimentally determined using a batch approach, and the results are compiled in Figure 3.2 and Table 3.2. The adsorption follows the same general trends (Figure 3.2, Table 3.2) in terms of levels of P sorption from the adsorption isotherms in Fig. 3.1b from 0-5 mM citrate, with a slight increase in P sorption from 0-5mM citrate, maintaining a stable pH. This is consistent with findings from (Duputel et al., 2013a). When adding citrate at low levels at pH 6, a portion of the soil's $CaCO_3$ is expected to dissolve, buffering pH and releasing Ca^{2+} that can react to form calcium phosphate minerals. When increasing the concentration of citrate in the control, depleted, and saturated soil, especially 10-50mM of citrate, it indicates that there is a significant decrease of adsorption of P onto the soil compared to lower concentration of citrate (0-5mM). With the nitric acid addition there is no significant difference among the level of acid added 0-50mM equivalent (Table 3.2). However, a significant decrease in P sorption is observed from 10-50 mM citrate in control, depleted and saturated soil compared

to nitric acid treated soil (Figure 3.2), implying effective competition between citrate and phosphate at these concentrations. To test whether the major role of citrate was that of a reactive ligand or proton source, nitric acid was used in place of citrate at equal molar levels; data shows approximately constant adsorption of P until 25-50mM of nitric acid, where, in contrast with citric acid, increased P sorption occurs.

By increasing citrate concentrations through additions to our soils the amount of adsorbed P decreases allowing for increased transport of P. XANES were analyzed in Figure 3.3, illustrating the amount of P present for all samples is 40-70% adsorbed with the remainder present as a calcium phosphate species. In all concentration levels from 0-5mM of citrate the effect on P sorption between the Ca treatments is similar, at 10mM citrate the changes become more pronounced, with the control having the most significant difference compared to the other Ca treatments. From 25-50mM Citrate the effect on all Ca treatments is the same, except for the nitric acid on the control soil. The Ca-depleted pretreatments led to slightly greater amounts of phosphorus adsorption complexes relative to calcium phosphates as indicated by a dampened feature around 2162 eV. This dampening indicates that soil pretreatments allow for different calcium phosphate species to form. XANES collected on solid residues from the citrate desorption study are shown in Figure 3.3 as a function of both treatment and citrate/nitric acid concentration, indicate small but consistent changes in speciation with the addition of citric acid over the controls. A simple two component model consisting of adsorbed phosphate and a Mg-substituted brushite ($Mg/CaHPO_4*2H_2O$) mineral phase can describe the dataset with equal goodness of fit to more complex models, and so it was used for this analysis. These two phases were also the dominant two species observed in field-scale analysis of soils from the site after longer term reaction at pH 6.5 (Siciliano et al., 2016). In general, a few trends can be observed from the XANES dataset. First, for the samples without citrate (labeled 0 mM), the relative proportion of brushite increased in the order depleted < control <saturated, which is consistent with the fact that the system was roughly at saturation with respect to brushite in the control. In the control, depleted, and saturated soil treatments, the major effect of increasing citrate concentrations is a decrease in adsorbed PO_4. In contrast, the nitric acid samples show at dramatic shift to increased adsorbed PO_4 with increasing nitric acid. Note that the 0mM nitric sample is a digital reproduction of the control 0mM citrate sample rather than a separate sample that was added to Figure 3.3 to aid visual comparison.

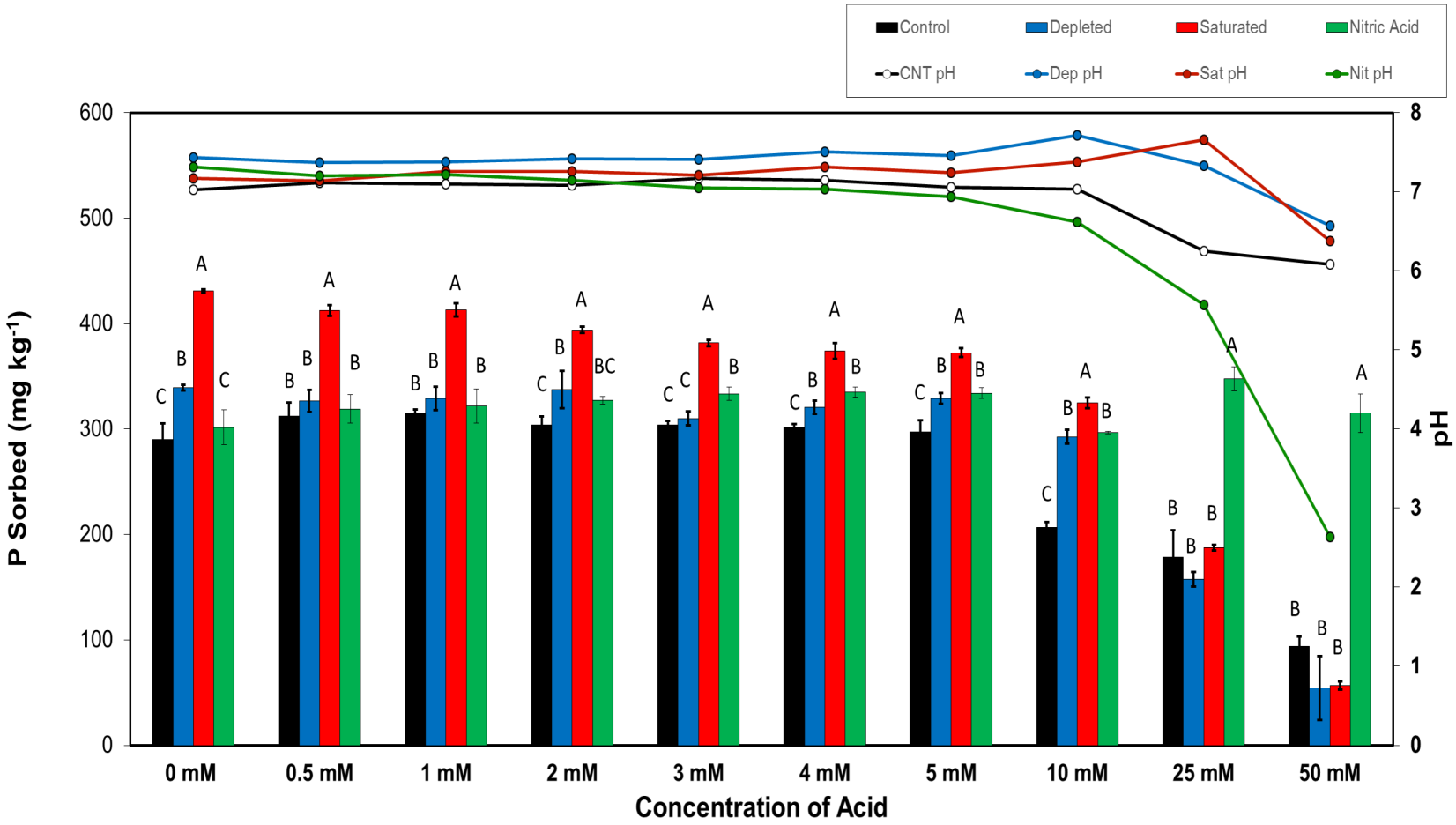

Fig. 3.2: Phosphorus sorption and solution pH following the addition of citrate (to control, Ca-depleted, and Ca-saturated soils) and the addition of nitric acid (to control soil). Mean values of P sorption (bar graph) with the same letter by acid concentration are not significantly different at $p < 0.05$. Error bars are one standard deviation ($n = 3$).

Table 3.2: Effect of acid addition on P adsorption: citrate on control soil, Ca-depleted soil, Ca-saturated soil; and nitric acid on control soil.

Concentration of acid	Control	Depleted	Saturated	Nitric acid
	P sorbed (mg kg^{-1})			
0 mM	290 A¶	339 A	431 A	302 BC
0.5 mM	312 A	326 AB	412 B	319 ABC
1 mM	314 A	329 A	413 B	322 ABC
2 mM	304 A	337 A	394 C	327 ABC
3 mM	304 A	310 AB	382 CD	333 AB
4 mM	302 A	321 AB	374 D	335 A
5 mM	297 A	329 A	372 D	334 AB
10 mM	206 B	292 B	325 E	296 C
25 mM	178 B	157 C	187 F	347 A
50 mM	94 C	54 D	57 G	315 ABC

† Means within columns followed by the same letter are not significantly different at $p < 0.05$.

3.6 Discussion

When all analytical sources are considered, it becomes clear that both calcium and citrate play an integrated role in P sorption on to the soils in this study. Across the treatments, a maximum equilibrium solution of 1mM P resulted in an adsorption plateau; exceeding this P level likely resulted in precipitation. This is consistent with the fact that a concentration of 1mM P was chosen in field-scale experiments outlined by (Siciliano et al., 2016) based upon geochemical modeling suggesting that this was the maximum P that can be added without exceeding saturation indices for calcium phosphate minerals at pH 6.5. Additional Ca would therefore be expected to lower the solubility of P in this system. The Ca-modified soils (both saturated and depleted) had higher P loading (adsorption maximum, Smax) capacities than did the unmodified soil; this could be due to breaking up of soil aggregates during the pretreatment to improve accessibility of surface sites, or by directly changing soil Ca levels to allow for more P sorption through possible mineral precipitation. The modification of Ca levels in soils were effective based upon elemental analysis and XRD where a decrease in calcite and increased amorphous content were observed after treatment. Deductions from BET indicate that soil Ca processing decreased soil external surface area in comparison to the control, however as evident in Figure 3.2, and Table 3.2 this does not

appear to be the contributing factor in P sorption and influences of Citrate on the amount of P sorbed onto the soil.

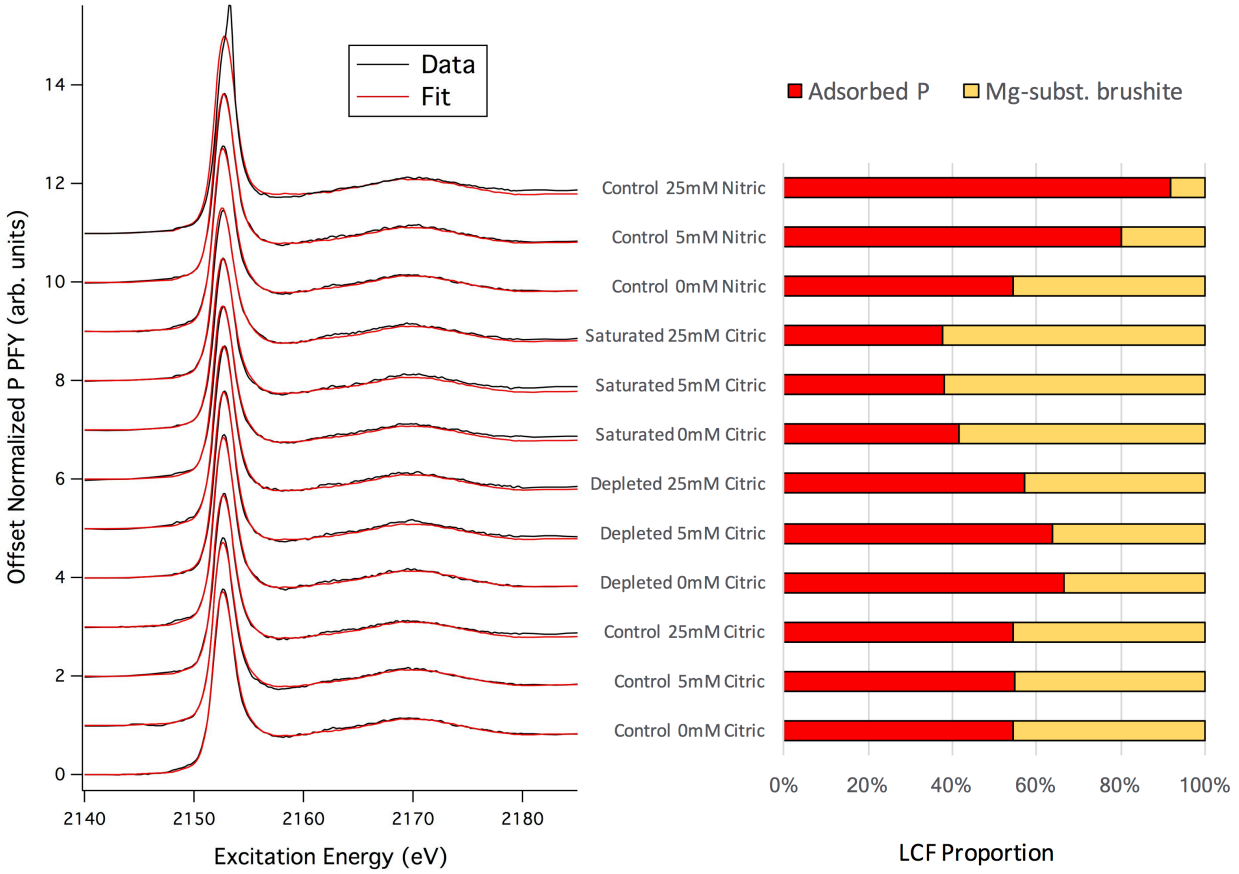

Fig. 3.3: (a) Phosphorus K-edge x-ray absorption near edge structure (XANES) spectra (black lines) of selected soil samples (control, Ca-depleted, and Ca-saturated soils containing varying concentrations of citrate) with a two-component linear combination fit (LCF, red lines) model overlain over plotted data. (b) Results of linear combination fit from the different treatments with varying concentrations of citric and nitric acid, showing the relative changes in P speciation among soil samples.

Next, the effect of adding a low molecular weight organic acid to samples with 1mM P adsorbed was tested in a separate experiment. At lower citrate concentrations 0-5mM, citrate does not have a large effect on P adsorption, and large amounts of citrate (more than 10mM) were required to decrease P retention on all soil treatments. This can be explained by the dissolution of carbonates in the soil that requires substantial quantities of citrate to exceed a calcareous soil buffering capacity. At higher citrate concentrations, this buffering effect is overcome and citrate acts in competition with P for adsorption sites (Geelhoed et al., 1998; Mikutta et al., 2006). To verify the role of carbonate buffering on the system, nitric acid was added in the control soil to determine its effect as a proton source that does not have the ability to either chelate Ca in solution or

competitively desorb phosphate. The addition of nitric acid above 10mM, as with citric acid, lowered the pH of the system, strongly implying that this corresponds to the soil's buffering capacity. However, since nitric acid is a strong acid, the pH decrease at levels of 25 or 50 mM was much larger but did not result in a larger corresponding desorption of P. This suggests that the major effect of nitric acid may be to dissolve phosphate-bearing minerals. This is unsurprising, as both brushite and carbonate phases are both unstable below pH 6. This dissolution without large losses in sorption may be due to enhanced adsorption on Al and Fe oxide surfaces at acidic pH. When considered with the XANES data that demonstrates an increase in the relative proportion of adsorbed P above 10mM, it can be concluded that competitive adsorption between citrate and P is the primary mechanism of interaction, and that this competition is most effective at desorbing P after soil carbonates have dissolved. This may explain some of the contradictory results in the literature, as there would be a range of buffering capacities in soils of different genesis and pH.

3.7 Conclusions

In conclusion, the results of this study provided insight into the roles of excess Ca and Mg in the speciation of phosphate in model calcareous soils. When compared to the controls, the addition of excess Ca (Ca saturation treatment) tended to produce a higher relative proportion of brushite-like calcium phosphates, whereas the treatment with an excess of Mg and reduced Ca (Ca depletion) had a higher relative proportion of adsorbed phosphate. When low amounts of citrate (0-10 mM) were added to these soils, very little change in soil pH, the quantity of phosphate sorbed, or its chemical species were observed. This suggests that, in calcareous soils, there is considerable overlap between buffering capacity and phosphate/citrate interactions. When citrate levels were increased to between 10-50mM, a significant reduction in the quantity of phosphate sorbed and a reduction in the relative abundance of adsorbed P was observed. This suggests that, for these experimental conditions, competition for adsorption sites may be the most important mechanism of interaction between citrate and phosphate. In short, the presence of soil carbonates tend to negate any acidification from citrate addition and buffers the soil at a neutral state. With the addition of citrate at high levels, we exceed this pH buffering capacity and observe a decrease not only in pH but also in P sorption. Future research should investigate the microbial implication of this and determine whether microbial communities are affected positively or negatively by the increase in both P and organic C (via citrate) and the contaminant decrease in pH.

4. EFFECTS OF CITRATE UPON THE RATES AND MECHANISMS OF PHOSPHATE ADSORPTION AND DESORPTION ON A CALCAREOUS SOIL

4.1 Preface

The mechanisms of P transport and how calcium and citrate can influence transport rates is key to not only hydrocarbon in-situ bioremediation but also many other soil chemical processes. Site soils were taken from the Broadway site in Saskatoon Saskatchewan, where soils were collected via coring. Taking the information in Chapter 3 about steady-state behavior, this chapter utilized a combination of flow-through kinetics experiments, X-ray absorption spectroscopy (XAS), and principal component analysis (PCA) of molecular and wet chemical data to visualize these relationships are defined and illustrated. This chapter covers the information gained from the kinetics reactions and how the data was modeled and what this model explains, allowing for future remediation of hydrocarbon contaminated sites. This paper is published in December 2018 issue of SSSAJ.[2]

4.2 Abstract

Adsorption at the soil/water interface is a fundamental chemical process that often controls retention of compounds in soils. The rates of adsorption therefore have a vital role in fate and transport of phosphate in the soil environment. In this study, the effects of a range of organic acid (citrate) levels and soil Ca levels upon phosphate (PO_4^{3-}) adsorption and desorption were investigated in detail using a combination of laboratory (miscible displacement kinetics) and synchrotron-based X-ray absorption spectroscopy (XAS). A pseudo second order kinetics model was applied to the data that provided good-fits of the entire datasets (both adsorption and

[2]David Bulmer; Jordan Hamilton; Gourango Kar; Gurbir Dhillon; Bing Si; Derek Peak. (2018). EFFECTS OF CITRATE UPON THE RATES AND MECHANISMS OF PHOSPHATE ADSORPTION AND DESORPTION ON A CALCAREOUS SOIL. *Soil Science Society of America Journal.* 10.2136/sssaj2018.09.0323

desorption) with a single set of equations. In general, it was observed that citrate directly competed for adsorption sites in soils with reduced levels of Ca, but that in soils high in labile Ca, citrate was not competitive in desorbing P. The most likely explanation for this is that citrate instead chelates with Ca to form soluble complexes when an excess of Ca is present.

4.3 Introduction

In natural environments, the most common form of phosphorus (P) is phosphate (PO_4^{3-}), which is highly bioavailable when dissolved in solution (Siciliano et al., 2016). In soil systems, dissolved PO_4 is inhibited through strong partitioning of PO_4^{3-} via sorption processes such as adsorption on mineral surfaces and precipitation of phosphate-bearing minerals. In calcareous soils, characterized by neutral pH and high calcium carbonate content, this typically leads to formation of sparingly soluble calcium phosphate minerals that decrease bioavailability and greatly reduce P transport (Tunesi et al., 1999; Cao and Harris, 2008; Manimel Wadu et al., 2013; Siciliano et al., 2016; Bulmer et al., 2017; Hamilton et al., 2018). The extent that Ca-P minerals limit solubility depends strongly upon the crystal structure of the mineral phase, which largely depends upon solution pH (Peak et al., 2012). A general rule is that the increase in pH from 6 to 8 causes a decrease in the solubility of calcium phosphate, as the thermodynamically-favored phase shifts from monocalcium phosphates, to brushite (dicalcium phosphate dihydrate), to hydroxyapatite (Dorozhkin, 2011).

In addition to Ca-P mineral formation, phosphate is also strongly attracted to calcium-rich carbonate minerals via the process of adsorption (Gätcher et al., 1998; Tunesi et al., 1999; Cao and Harris, 2008; Oburger et al., 2011). Aluminum and iron oxide minerals also have a strong adsorption affinity for PO_4^{3-}; the mechanism of PO_4^{3-} adsorption on both carbonate and oxide surfaces is ligand exchange, wherein a surface water or hydroxyl group is displaced by PO_4^{3-} and a covalent bond forms at the mineral surface. Competition for adsorption sites can result in either competition with other anions for a finite number of surface sites, or alternatively ligand-promoted dissolution of the minerals which eliminates solid phase and therefore surface sites of the mineral altogether (Strawn and Sparks, 2000; Penn et al., 2013).

Given the central role of adsorption to phosphate retention in soils, one chemical approach to enhancing dissolved PO_4^{3-} is to alter the chemical conditions of the system via addition of another competitive ligand reactant. A common candidate is addition of low molecular weight organic acids (LMWOA), which can catalyze the dissolution of phosphate and carbonate minerals via

chelation of Ca, directly compete with phosphate for adsorption sites, and can change the aggregation of soils which also influences sorption (Bolan and Naidu, 1994; Wang et al., 2015). One common LMWOA for increasing P solubility is citrate. However, there are conflicting results in the literature regarding the effectiveness of LMWOAs such as citrate on P solubilization (Geelhoed et al., 1998; Duputel et al., 2013a; Siciliano et al., 2016; Bulmer et al., 2017) . Specifically, some studies of citrate/P interaction show that citrate additions enhance P mobility whereas sometimes P mobility is decreased (Bolan and Naidu, 1994; Gao et al., 2010; Duputel et al., 2013b; Siciliano et al., 2016). For soils with high clay content, the mechanism for the decrease in P mobility upon citrate addition has been proposed to be an increase of surface negative charges, leading to an increase in the adsorption of Ca^{2+} ions onto the clay minerals (Duputel et al., 2013a). In a recent study (Bulmer et al., 2017), we investigated the steady-state interactions of Ca, PO_4^{3-}, and different amounts of citrate in a calcareous subsoil. The soils contained high levels of both Ca^{2+} and Mg^{2+} cations that can potentially react to form phosphate minerals, and high levels of carbonates in the soil that can serve not only as adsorption sites but also to buffer the soils from changes in pH. The addition of a LMWOA such as citrate will be buffered from acidification by soil carbonate mineral dissolution, releasing Ca^{2+} and thus promoting the formation of Ca-P phases, hindering P mobility (Tunesi et al., 1999; Cao and Harris, 2008; Stoner et al., 2011; Penn et al., 2013). A pre-treatment experiment was devised to either remove soil Ca and increase Mg concentrations, or increase Ca levels beyond their current levels and saturate exchange sites. This produced three treatments: untreated (control), Ca saturated, and Ca depleted (Wandruszka, 2006; Manimel Wadu et al., 2013; Bulmer et al., 2017). The results helped to (a) illustrate the roles of excess Ca and Mg in the speciation of phosphate and (b) demonstrate the influence of citrate on P adsorption. When comparing the control soil to the Ca saturated treatment, a higher relative proportion of brushite-like calcium phosphates formed, which differed from Ca depleted (excess Mg) treatment, which had a relatively higher proportion of adsorbed phosphate. Results indicated that the effectiveness of citrate on P was more pronounced after the addition of citrate exceeded 10 mM (10-50 mM), at which point the amount of P adsorbed was significantly decreased. Overall, this study proposed that competition for adsorption sites was the most important interaction between citrate and phosphate at steady-state.

Reactions in soils are dynamic and may not reach equilibrium; instead many sorption reactions are time dependent (Sparks, 1989). Performing kinetics experiments and determining rates of adsorption and desorption are essential for the thorough evaluation of sorption in soils (Ho and

Mckay, 1998a; Ho and McKay, 1999; Azizian, 2004; Riahi et al., 2013; Rout et al., 2015; Chen et al., 2017). Accordingly, the design of this study was to test the importance of varying Ca and citrate levels upon rates of PO_4^{3-} adsorption and desorption in a calcareous soil. Using methods developed by Sparks (Sparks, 1989) and refined in our previous work (Eick et al., 1999; Peak et al., 2012), a thin disk (miscible displacement) experiment was designed to determine the rates of P adsorption and desorption on our samples.

4.4 Materials and Methods

4.4.1 Soil Samples and Pretreatments. The soil was collected as part of a previous study (Siciliano et al., 2016; Bulmer et al., 2017), this soil was a consolidated subsoil from 3-6m depth of a Western Canadian contaminated site. The soil classifies in the Canadian system as a Regosol, which is a clay loam soil consisting of 27% sand, 41% silt, and 32% clay. With a bulk density of 1.3g cm^{-3} a pH of 7, and a TIC of 1.02%, soil samples for laboratory experiments were collected as outlined by (Siciliano et al., 2016 and Bulmer et al., 2017), using a push core drill to collect samples at two different sites (Broadway South Side, and Broadway West Side) in Saskatoon, Saskatchewan, Canada. Soils were hydrocarbon contaminated for several years and frozen for 4-6 months a year. After soils were collected, they were stored at -20°C. After initial screening to verify that the texture (Djodjic, 2004; Zheng and Zhang, 2011), and basic properties of the individual samples were similar, all subsamples of the cores were consolidated to produce a single bulk sample representative of the site. This was done out of necessity to produce enough soil for this study. The consolidated soil is a clay loam (27% sand, 41% silt, and 32% clay) with a bulk density of 1.3 g cm^{-3}.

Two experimental manipulations of the consolidated soil (which hence is referred to as control) were performed to produce a calcium-depleted and a calcium-saturated soil. The method for soil treatment is outlined by (Siciliano et al., 2016 and Bulmer et al., 2017). Briefly, the control soil was not treated, the Ca-depleted soil was prepared by adding deionized water to a 1:1 soil slurry, and further reducing calcium concentrations in the soil through a 1.0 M $MgCl_2$ solution while also adjusting pH to 6.5 with 1.0 M H_2SO_4, which was then centrifuged and washed three times, therefore removing excess ions as well as $CaSO_{4(s)}$. The soil was then added to a 1.0 M $MgCl_2$ solution in a 1:4 ratio, allowed to react overnight with mild stirring, then washed three times with deionized water. This entire process was repeated three times to exchange bound (adsorbed) Ca^{2+} with Mg^{2+}. The Ca-saturated soil was made through the addition of 1 M $CaCl_2$ in a 1:4 ratio and

stirred with a magnetic stir overnight, then washed via centrifugation three times with double deionized water (DDI) to remove excess ions. This process was repeated three times to increase the amount of bound Ca^{2+} in the soil. All soils including control were then oven dried at 80°C to remove any excess moisture before experimental use.

4.4.2 Soil Surface Area. A consolidated sample from each soil treatment was analyzed for external surface area via 3 point BET (Brunauer-Emmett-Teller) surface area analyzer using an ASAP 2020 by Micromeritics as part of a previous study (Bulmer et al., 2017). The surface area of the soils were 31.5, 23.4, and 19.1 $m^2 g^{-1}$ for the control, depleted, and saturated soils, respectively.

4.4.3 Miscible Displacement Kinetics Experiments. Since previous studies (Siciliano et al., 2016) identified preferential flow paths through large fractures as the dominant transport regime in glacial till soils, we applied a miscible displacement approach to study reaction kinetics. With this approach, it is expected that some mass transport limitations through the film of surface water is expected to be a limit on the rate of reactions, which is also true for preferential flow regimes. Specifically for these experiments, 0.3 grams of soil were applied to a filter disk using a 47 mm filter holder. The tank solution of 0.011 M $MgSO_4$ and 1 mM PO_4^{3-}, in DDI water, was passed through the filter with soil at a rate of 1 ml min^{-1} and collected with a fraction collector at intervals of 0.5 to 1 minutes. Since our kinetics experiments were conducted with a constant flow rate, we were able to compare the relative changes to the different treatments. The resulting adsorption kinetics experiment was modeled to estimate P adsorption rates on the soil. After 130 minutes, a desorption solution was applied to facilitate P release from the soil. Desorption was performed using just 0.011 M $MgSO_4$, 1 mM P and citrate, or $MgSO_4$ and citrate solution in water. The final measurement was taken on the Seal Analytical Auto Analyzer (Seal Analytical, Germany), through the colorimetrical analysis using a molybdenum blue method (Murphy and Riley, 1962).

4.4.4 Kinetic Modeling. Multiple kinetic models were applied to the dataset, including 1st order, Elovich, film diffusion, and pseudo second order models. The first three models could not describe the adsorption or desorption process with a single equation (data not shown), and instead required multiple steps to fit the results, which is typical of the literature for experiments involving soils. In contrast, the pseudo second order model (PSOE) was capable of modeling all the thin disk kinetics data with high R^2 values. The premise of the PSO model is that the overall rate-limiting factor is chemi-sorption (ligand exchange) reactions (Ho and McKay, 1999; Plazinski et al., 2013). We utilized the PSO model as derived by Ho and McKay (1999) without modification to model adsorption and desorption kinetics in Microsoft Excel (2016).

4.4.5 P K-edge XANES spectroscopy. P K-edge X-ray absorption near edge structure (XANES) spectra were collected on the Soft X-Ray Micro-characterization Beamline (06-B1-1) of the Canadian Light Source in partial fluorescence mode, using 4 element solid state detector (Bruker). Data was processed and analyzed using the Athena software package (Ravel and Newville, 2005). Linear combination fitting (LCF) was performed on adsorption samples using a large library of high quality phosphate reference compounds that included adsorbed phosphate, phosphate minerals with a range of cations, and organic phosphates (Kar et al., 2017; Hamilton et al., 2018). Standards were diluted in boron nitride to avoid saturation effects on XANES from self-absorption. The principles used to develop the LCF model was based upon (a) using the minimum number of standards for the dataset to reproduce the samples with acceptable goodness of fit (quantified via Athena's reduced chi squared). (b) Preference was given to phosphate minerals that were stable at the pH of the soils over other minerals when similar fits were achieved.

4.4.6 Statistical Analysis. All adsorption data on the effect of treatments and citrate were subjected to analysis of variance (ANOVA) using PROC MIXED in SAS (Statistical Analysis System, Version 9.4 for Windows™; SAS Institute, Cary, NC). The denominator degrees of freedom method (*ddfm*) option will use the Satterthwaite method. Multi-treatment comparisons of variables are made using the Tukey's studentized range test method of mean separation at the probability level of $P \leq 0.05$ to establish statistical significance, where pdmix800 SAS macro (Saxton, 1998) was used to assign grouping. Principal component analysis (PCA) was performed on the relative proportions of P minerals, as determined through the LCF of XANES spectra, in addition to the model output from pseudo-second order equation (q_e and k_2). Data were mean-centered, scaled and projected using first two principal components such that their inter-point distances corresponded to the differences in P speciation (adsorbed, apatite, and newberyite) and kinetics (q_e and k_2).

4.5 Results

Changing the geochemical properties with soil pre-treatments did have an effect on P adsorption. All the treatments appear to show the same general adsorption and desorption pattern (Figure 4.1), with soil Ca modifying the total amount of phosphate adsorbed and retained. Most of the adsorption occurs by the first 35 minutes with minor additional adsorption continuing until 60 minutes. As illustrated in Table 4.1, the amount of adsorbed P differs greatly among the treatments, where the depleted and calcium saturated soil have significantly higher adsorption capacity

compared to control treatment. Interestingly, specific surface area of the three soils decreasing from control soil > Ca depleted > Ca saturated, implying the mechanism of increased adsorption is not a function of surface area. All three soil treatments had a P desorption rate that was slower than adsorption, which is typical for phosphate reactivity in soils. The calcium saturated soil showed the highest adsorption (Q_e) and second order rate constant of sorption (k_2), but no significant difference was observed among the treatments in terms of adsorption rates (Table 4.1). In general, a PSO kinetics model describes both the adsorption and desorption well with a small number of floating parameters. Results indicate that addition of citrate decreased the amount of P retained by the soils most strongly in the control soils (Figure 4.1), had little effect in the Ca depleted soils, and decreased desorption in the Ca saturated soils. For the adsorption portion of the experiments, there was a significant difference among the equilibrium P sorption values across all three treatments with the two treated soils having significant increases in P sorption maxima relative to the control soil. However, there was no significant difference in pseudo-second order rate constants for the treatments, which may imply that diffusional processes rather than chemical reactions might limit sorption kinetics in this experimental system. The PSO model was also applied to the desorption experiments, and the results were analyzed via ANOVA and tabulated in Table 4.2. Generally, it was observed that citrate has some effect upon both the amount of phosphate desorbed and the rate of desorption, with enhanced phosphate release typically corresponding to increasing citrate levels. There are some significant differences in both the final phosphate concentrations and the PSO reaction rates, but the variation isn't clearly a result of either pre-treatment or citrate concentration. For example, the depleted soil with no citrate in the desorptive solution is significantly different then saturated soils with citrate added. The control with 25 mM citrate was significantly different from depleted soils with no citrate in the desorption agent. For the Ca-saturated soils, there was a significant difference between no citrate and the two citrate treatments, with citrate treatments resulting in less desorption and slower PSO rate constants.

P K-edge XANES spectroscopy. It is difficult to quantitatively compare the XANES samples in Figure 4.2, since each spectrum is the result of a separate replicate and soil heterogeneity can play a role in the observed speciation. However, there are some interesting trends in the results. In general, LCF is consistent primarily with a three-component mixture of adsorbed P, crystalline calcium phosphate, and a magnesium phosphate. In the case of adsorbed phosphate (70-90% of

total), this species is a logical consequence of the reactive transport of aqueous P through the system and suggests that the dominant sorption mechanism for this short-term experiment is adsorption. The apatite component (6-24% of the LCF) was likely present in the soil prior to the kinetics experiment based upon the fact that it is a much larger component of the untreated control than in the treated samples, and that it is virtually eliminated in the Ca depletion sample. A small newberyite ($MgHPO_4*xH_2O$) component is present in all samples; this does not appear to be strongly influenced by treatment since the sample with the highest Mg levels (depleted) has the lowest contribution from newberyite. The major trend in these adsorption samples is that the depletion treatment reduced Ca-phosphate minerals and increased the relative fraction of P that was adsorbed. This is consistent with the findings of previous steady-state measurements of P speciation with LCF (Bulmer et al., 2017). The three soil treatments responded differently to desorption. In the case of control soil, desorption with background electrolyte (0mM citrate) and citrate (25mM citrate) both showed a relative loss of apatite. In the case of desorption with citrate, the control sample showed a loss of adsorbed P followed and an increase in newberyite. This is consistent with citrate primarily behaving in a role of competition with phosphate for adsorption sites on this soil.

For both the depleted and saturated soil treatments, desorption with background electrolyte (samples with 0 mM cit) did not markedly change the relative abundance of any of the species; this suggests that the release was due to adsorbed and mineral forms of P equally. In the presence of citrate (samples with 25 mM cit suffix), the depleted samples had a large reduction in apatite and an increase in the fraction of newberyite whereas the adsorbed component remained consistent at 70-75%. This suggests that in contrast with the control soil, citrate in the depleted soil primarily functioned to dissolve calcium-bearing phases rather than directly competing with phosphate for adsorption sites. In the saturated soil, the effect is somewhat different, where citrate led to increased fraction of adsorbed P and a reduction in both apatite and newberyite.

Table 4.1: Properties of PO_4 adsorption parameters for the three calcium treatments (actual) versus their 2nd order kinetics model outputs.

Soil	Experimental		2nd Order Model			
	$q_e(\mu g\ g^{-1})$		$q_e(\mu g^{-1})$	k_2 (g $\mu q(p)^{-1}$ min^{-1})		R^2
Control	740.9	c¶	769.2	5.1E-04	a	0.999
Depleted	1075.6	a	1000.0	3.6E-04	a	0.998
Saturated	1180.8	b	1111.1	3.4E-04	a	0.999

¶ Means with the same letter in the same column are not significantly different at $p < 0.05$.
q_e is defined as maximum adsorption values, k_2 is the rate constants of the Pseudo second order model.

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of desorption, with enhanced phosphate release typically corresponding to increasing citrate levels. There are some significant differences in both the final phosphate concentrations and the PSO reaction rates, but the variation isn't clearly a result of either pre-treatment or citrate concentration. For example, the depleted soil with no citrate in the desorptive solution is significantly different then saturated soils with citrate added. The control with 25 mM citrate was significantly different from depleted soils with no citrate in the desorption agent. For the Ca-saturated soils, there was a significant difference between no citrate and the two citrate treatments, with citrate treatments resulting in less desorption and slower PSO rate constants.

Table 4.2: Effect of Citrate addition on P desorption on the control, depleted, and saturated treated soils. Experimental (actual) is compared with the output from the 2nd order kinetics model.

Citrate	Soil	Experimental		2nd Order Model			
mM		q_e (µg g^{-1})		q_e (µg g^{-1})	k_2 (g µq(p)$^{-1}$ min^{-1})		R^2
0		595.8	de¶	587.1	8.8E-04	abc	0.999
5	Control	475.2	e	468.3	1.0E-03	ab	0.999
25		470.1	e	455.6	5.1E-04	bc	0.997
0		700.3	cd	693.9	1.0E-03	a	0.998
5	Depleted	825.4	abc	815.5	7.2E-04	abc	0.998
25		758.7	bcd	754.7	1.4E-03	abc	0.999
0		677.8	cd	670.8	1.1E-03	a	0.999
5	Saturated	956.5	a	938.5	4.1E-04	c	0.995
25		891.0	ab	884.4	3.4E-04	c	0.997

¶ Means with the same letter in the same column are not significantly different at $p < 0.05$.
q_e is defined as maximum adsorption values, k_2 is the rate constants of the Pseudo second order model.

Changing the geochemical properties with soil pre-treatments did have an effect on P adsorption. All the treatments appear to show the same general adsorption and desorption pattern (Figure 4.1), with soil Ca modifying the total amount of phosphate adsorbed and retained. Most of the adsorption occurs by first 35 minutes with minor additional adsorption continuing until 60 minutes. As illustrated in Table 4.1, the amount of adsorbed P differs greatly among the treatments, where the depleted and calcium saturated soil have significantly higher adsorption capacity compared to control treatment. Interestingly, specific surface area of the three soils decreasing from control soil > Ca depleted > Ca saturated, implying the mechanism of increased adsorption is not a function of surface area. All three soil treatments had a P desorption rate that was slower than adsorption, which is typical for phosphate reactivity in soils. The calcium saturated soil showed the highest adsorption (Q_e) and second order rate constant of sorption (k_2), but no significant difference was

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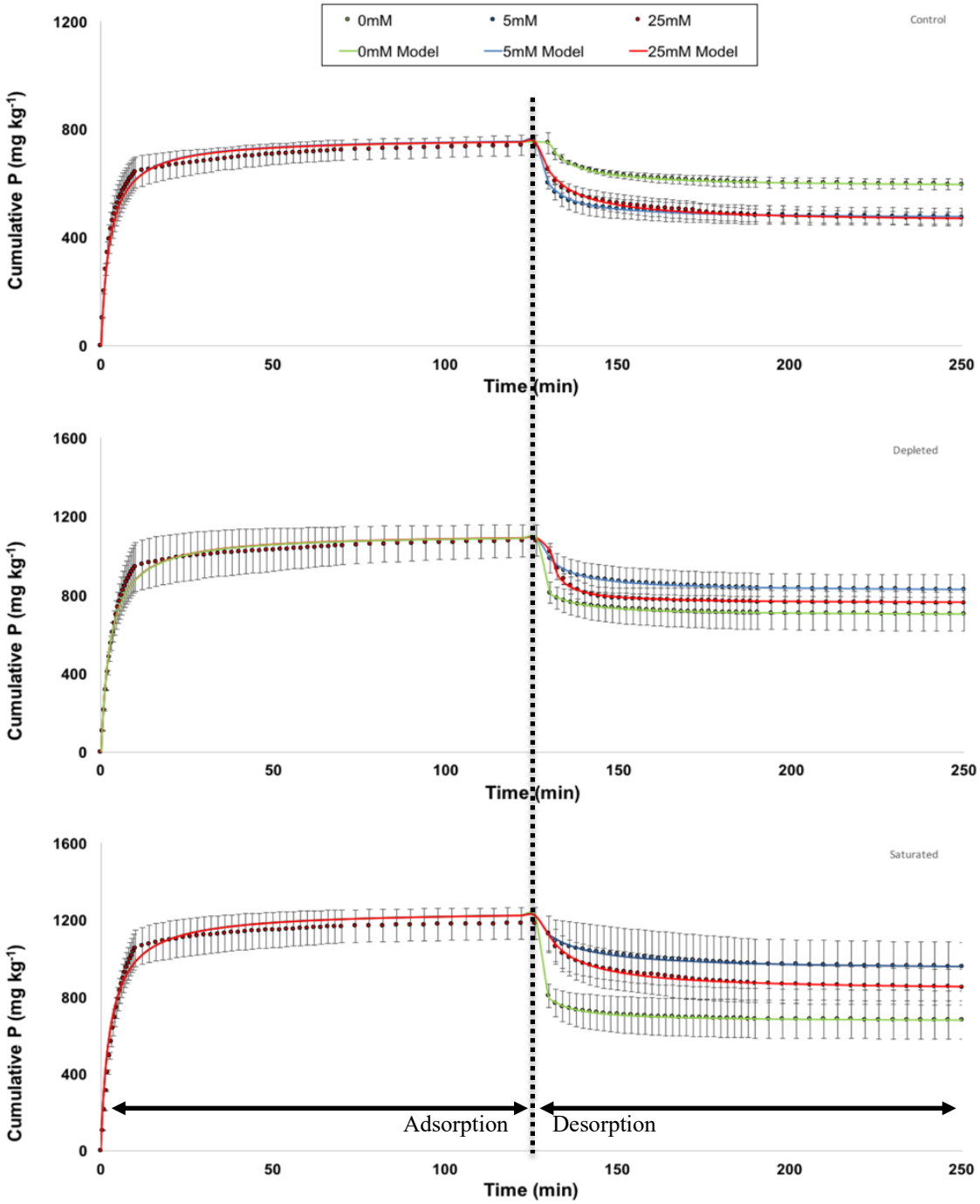

Figure 4.1: Adsorption/desorption of P on control soil using different citrate amendments, with pseudo 2nd order kinetics model applied. See adsorption/desorption values in table 4.1 and 4.2.

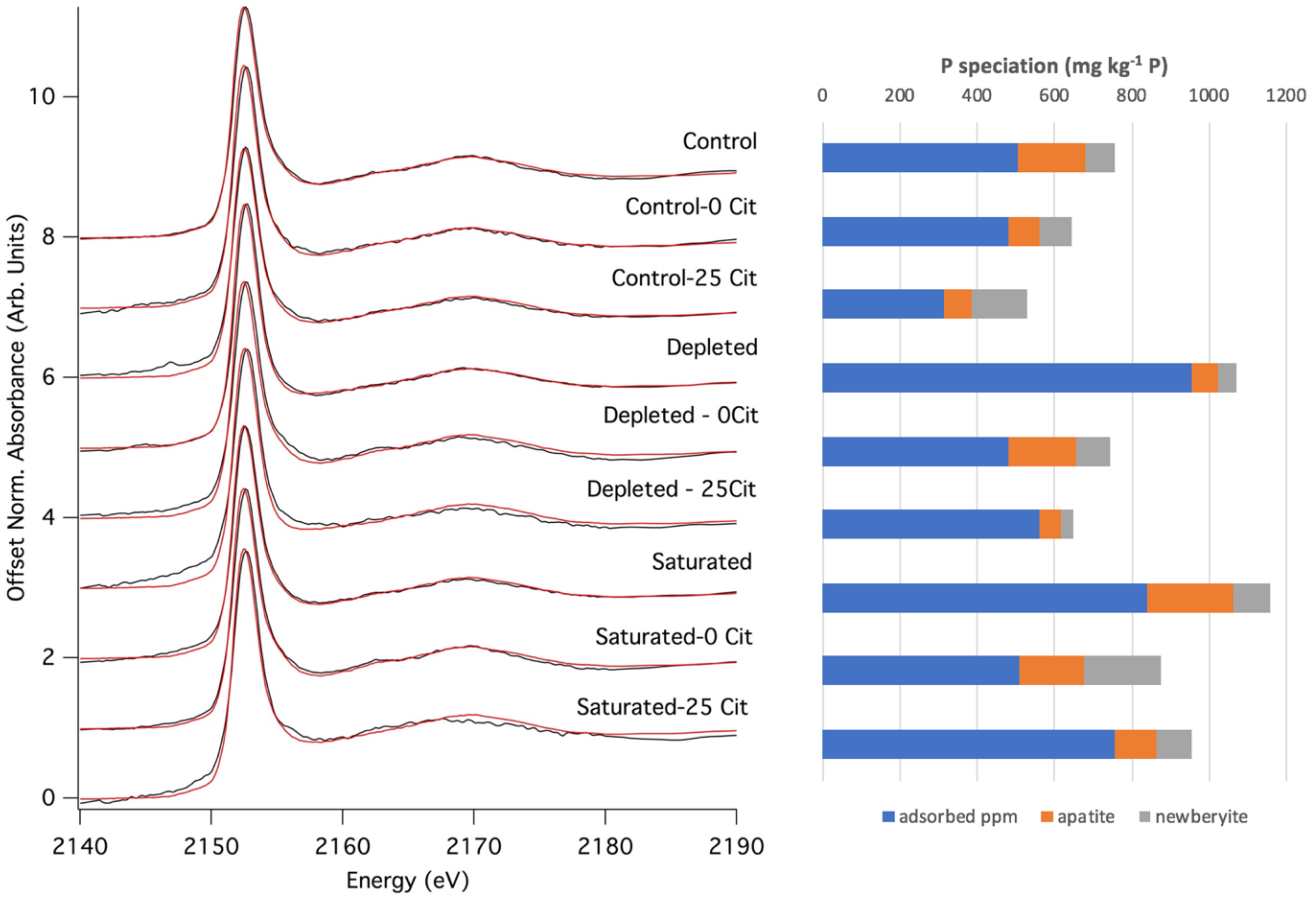

Figure 4.2: XANES data (black lines) and fits (red lines) for the samples at the end of adsorption or else at the end of desorption with 0 or 25mM citrate. LCF relative proportions can be found in Table 4.2.

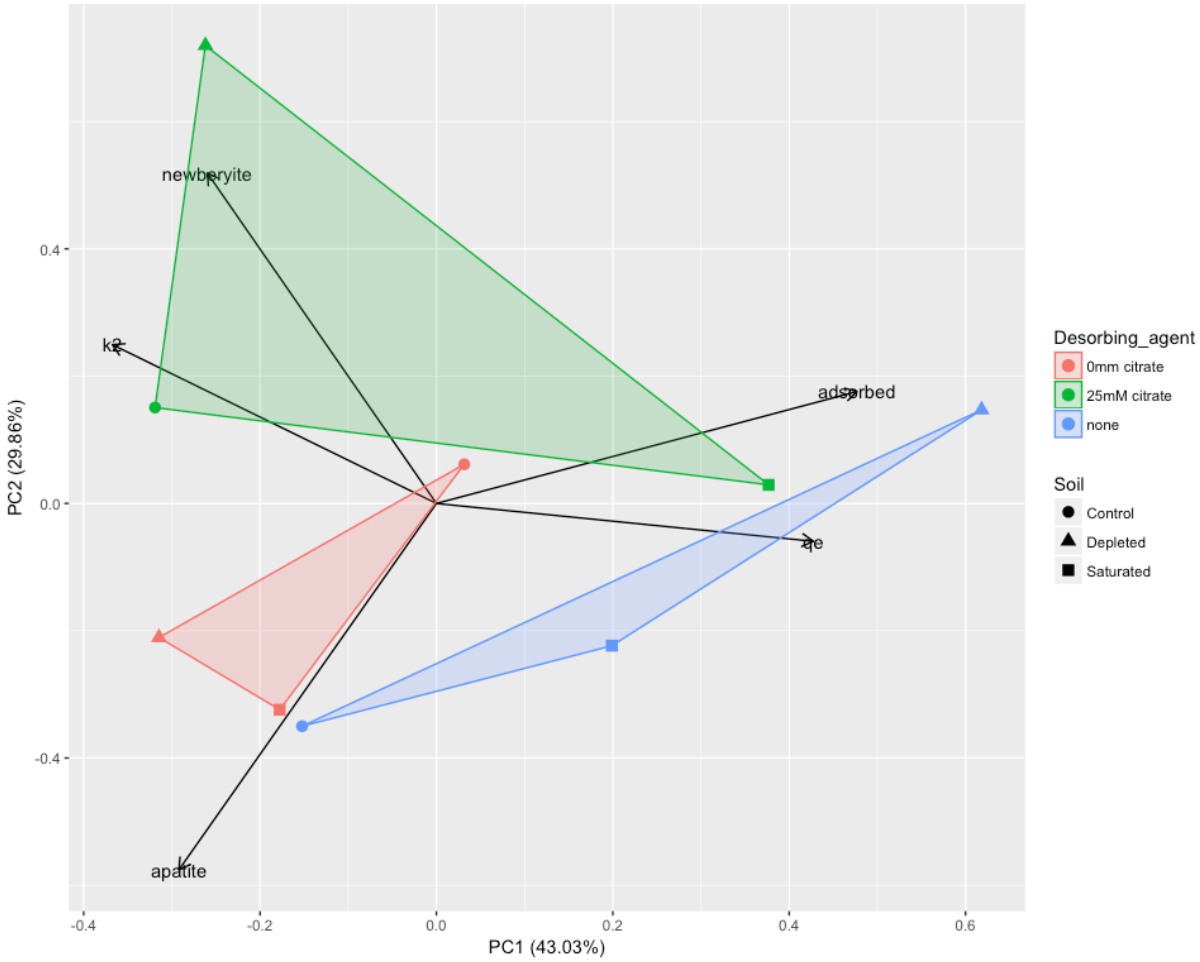

Figure 4.3: PCA cluster analysis of the LCF results clustered by desorbing agent. Vectors represent model output from the pseudo-second order equation (q_e and k) and speciation (adsorbed, apatite, and newberyite).

4.6 Discussion

When the kinetics modeling parameters and spectroscopic speciation are considered together, a clearer picture of phosphate/citrate interactions emerges. When the system has a large Ca content, citrate is less effective than the $MgSO_4$ background in desorbing phosphate, and the P species that are removed tend to be phosphate minerals rather than the adsorbed fraction, which remains fairly constant. In contrast, in the control soil the addition of citrate enhances desorption relative to the $MgSO_4$ background alone. The LCF results provide evidence that the principal species that is removed from the control soil by citrate addition is adsorbed P. To visualize these relationships in more detail, we performed principal component analysis on the LCF fit results, shown in Figure 4.3. Given the small number of samples (n=9), this is more exploratory than quantitative; multivariate approaches such as PCA are more valuable when larger datasets are analyzed. Nonetheless, it is clear that the samples clustered based upon the treatment (adsorption, desorption with 0 mM citrate, desorption with 25 mM citrate) thus elucidating its role in regulating P speciation. The samples lacking a desorbing agent showed higher fraction of adsorbed P in LCF, along with higher sorbed P at equilibrium (Q_e), which is expected. In the presence of citrate (samples with 25 mM citrate), the samples showed higher abundance of newberyite, with the exception of Ca-saturated soils, which showed higher abundance of adsorbed P. The loading vectors for newberyite and k_2 were closely associated. This may imply that dissolution of this phase, when present, is somewhat more rapid than dissolution of apatite or desorption of P. This may perhaps explain the depleted soil treatments, which are rich in Mg but showed no significant difference in desorption with varying citrate levels. It is possible that the newberyite phase that forms during the sorption experiment is rapidly dissolved when phosphate is no longer in the desorptive solution regardless of citrate.

The interaction between citrate and phosphate is completely different in the control and Ca-saturated soils, implying that the effectiveness of citrate as a competitive ligand depends upon Ca levels. In the system with a large amount of labile Ca^{2+}, citrate is ineffective at desorption. This may imply that instead of surface complexation, formation of aqueous Ca-citrate chelation complexes are dominant in this system. In systems where there is far less labile Ca (such as the control and depleted soils), citrate plays the role of competitive ligand and desorbs phosphate.

4.7 Conclusions

In conclusion, the results of this study provided new insights into the rates and mechanisms of phosphate-citrate-calcium interactions. The combination of flow-through kinetics experiments, pseudo second order modeling, and molecular-scale spectroscopic speciation can together elucidate kinetic processes in soils that cannot be understood with only one approach. Labile calcium levels appear to strongly influence citrate desorption effectiveness, with increased concentrations of Ca having a significant increase in the amount of P retained on the soil, with the addition of citrate having a statistically significant effect on P desorption amount from all the pre-treatments.

5. SYNTHESIS AND CONCLUSIONS

This thesis investigated the effects of Ca modification and the use of citrate on the effects of P transport through a calcareous cold soil that has been hydrocarbon contaminated. A key outcome of this thesis was to provide an effective model for citrate and P interactions that can be applied to future research and different contaminated sites in the Canada or the world. There are several important findings that corresponded to previous research as well as new discoveries, and the conclusiveness of the model used to accurately model the system. With this new information, recommendations to our site partner were made to further assist in remediation practices. Unsurprisingly, knowledge gaps and future research suggestions also arose from this work.

In chapter 3, it was observed that the amount of citrate used in combination with different amounts of Ca initially present can yield significant changes in adsorbed phosphate levels. The control soils (unmodified from sampling) showed the most significant response to citrate addition, with citrate levels greater then 5mM decreasing P sorption compared with the control. The citrate was successful in competing for binding sites on Ca-P minerals for the control soils, and this apparently reduced P incorporation. Higher amounts of citrate than expected were required to achieve the goal of 50% P inhibition. With lower levels of citrate added (<5mM) the effect on P was negligible, this is likely because of the site soil $CaCO_3$, levels that could interact with citrate without affecting P sorption capacity. This strongly suggests that, in the initial control soil, Ca buffering capacity exceeded citrate interactions at lower levels of citrate. By modifying the amounts of Ca in the soil, the amount of P adsorbed was also changed, further reinforcing the relationship between Ca and P in the soil system. Higher amounts of P were adsorbed based on the modification of Ca, this is likely due the modification of aggregates through the pre-treatment of the soils. The overall conclusion was that the soil's ability to retain calcium can be successfully overcome with the use of enough citrate (roughly 10:1). Since this study, a 10:1 citrate:phosphate amendment has been successfully employed not only at Broadway, but also at 9 other sites undergoing bioremediation. In all cases it was effective.

A key finding of chapter 4 was the ability to model the kinetic mechanisms involved in P-Citrate-Ca interactions with a single kinetic model throughout the entirety of adsorption and desorption with a minimal number of fitting parameters. When the system has a large concentration of Ca, citrate becomes a less effective desorptive agent of P, with the form of P removed being consistent with loss of P minerals instead of the adsorbed P fraction. In the unmodified control soil, there is a much stronger correlation with concentration of citrate to P

removal, this complements the findings in chapter 3, whereby increasing citrate we see a stronger return on P removal. With this information, a model to describe the system was built a pseudo-second order model. The success of citrate as a desorption agent is further reinforced from chapter 3, indicating the importance of Ca and the ability to overcome its binding effect with P. The primary outcome of the kinetic study is identifying that the rate limiting step is best characterized in preferential flow path dominated systems by film diffusion through pores and chemical reaction rates in a single composite pseudo second order equation, the output terms of which is completely compatible with geochemical transport models.

It is important to take note of the average yearly temperature for this site. The site's soils are frozen for approximately half the year, thus decreasing not only the rate of chemical reactions but also the biological activity. Since these soils are high in clay, they will form preferential flow paths during the freeze thaw cycles; the shape and connectivity of which depend upon moisture content. The amount of calcium carbonate from the parent material is clearly an important contributor in the speciation of Ca-P minerals, but it is also an important factor in the rates at which citrate can desorb P. The soil surface complexation exchange reaction is very important in P transport. The addition of citrate was designed to decrease the exchangeable sites for P to interact with both Ca and Mg. Overall the experiments provided an amendment ratio of 1:10 phosphate to citrate with a maximum phosphate level of 1mM (thus 10mM citrate) that significantly enhanced bioremediation at the Broadway site. This highlights the role of establishing adsorption maxima (via isotherms) and interaction mechanisms (via kinetics experiments and spectroscopy) for field-scale projects. Rather than costly trial and error on site, the understanding and modeling of fundamental interactions could predict the performance of the biostimulatory solution.

However, there were some inherent laboratory research limitations that complicated scaling up our results to the field. Site variables that are difficult to reproduce in model experiments include heterogeneity of soils and variability in temperature and moisture could strongly influence real world results. The laboratory experiments as conducted could be considered a snapshot of a much larger environmental picture; capturing some conditions of time and place in the broader image of bioremediation. The systems in the field in contrast are very much large-scale and may have more complex interactions with the environment. Accordingly, the future direction of this research are an investigation of temperature and soil sand lens influences on the mobility of P and how both the soil conditions at different textures and temperatures influences the chemical reactions of both P and citrate.

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APPENDIX : SUPPLEMENTARY TABLES AND FIGURES

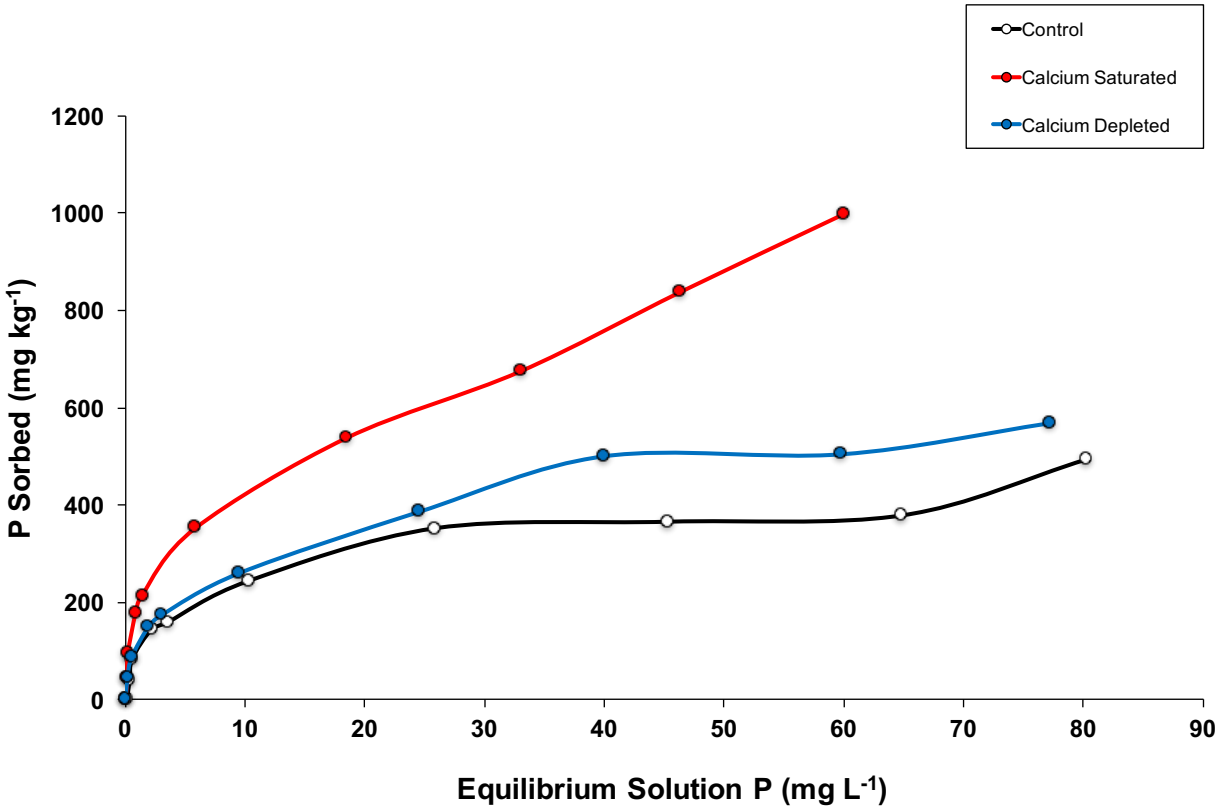

Figure A.1: isotherm of P adsorption on two soil treatments (Ca saturated, and Ca Depleted) and control soil at 25°C.

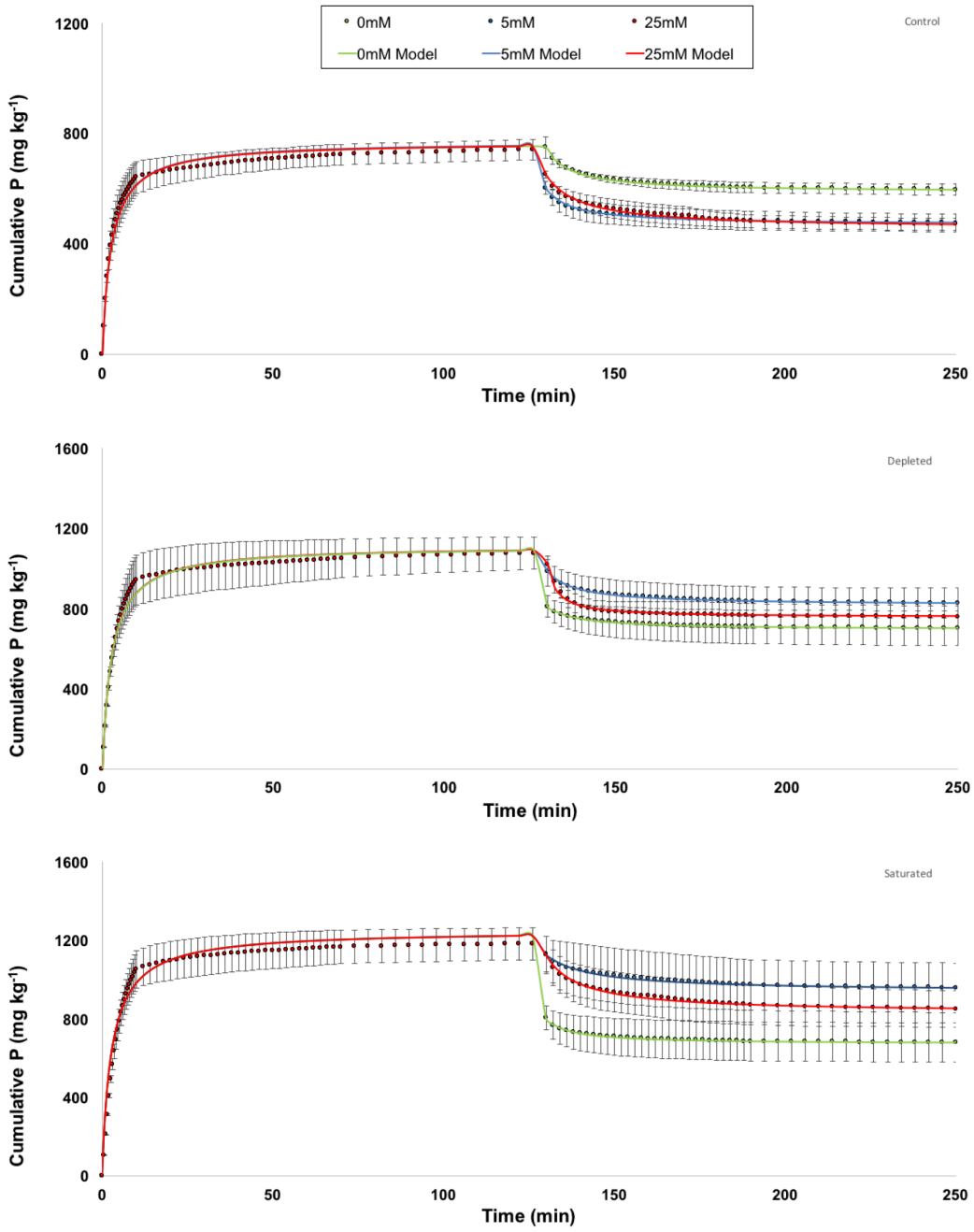

Figure A.2: isotherm of P adsorption and depletion on three soil treatments (Control, Ca saturated, Ca Depleted), using increasing levels of citrate as a desorption agent.

Table A.1 Total elemental concentrations, output from XRF

Element	Control		Ca Saturated		Ca Depleted	
	m/m%	Std Err	m/m%	Std Err	m/m%	Std Err
SiO2	23.53	0.24	26.98	0.26	27.34	0.26
Ca	1.58	0.05	1.83	0.06	0.53	0.03
Fe	2.81	0.07	2.93	0.07	2.44	0.07
K	1.26	0.05	1.31	0.05	1.34	0.05
Mg	0.68	0.03	0.65	0.03	1.04	0.04
P	0.10	0.01	0.10	0.01	0.10	0.01
Al	3.18	0.06	3.70	0.07	3.21	0.07
S	0.08	0.00	0.01	0.00	0.01	0.00
Mn	0.04	0.00	0.03	0.00	0.04	0.00
Sr	0.03	0.00	0.02	0.00	0.02	0.00
Ti	0.27	0.01	0.27	0.01	0.26	0.01
Zr	0.03	0.00	0.03	0.00	0.03	0.00
Zn	0.01	0.00	0.01	0.00	0.01	0.00
% Sum	46.3		48.2		46.8	

Table A.2 Soil characteristics for three soil types

Soil	pH (no Cit)	BET	BET (t-Plot)	Organic C	Inorgainic C	Total C	Ca (XRF)	Mg (XRF)
	Shaken 24 hours with mg	m≤/g			%		mg/kg	
Control	7	31.54	21.22	1.02	0.66	1.68	15800	6810
Ca Saturated	7.2	19.06	18.51	0.77	0.73	1.50	18300	6460
Ca Depleted	7.4	23.35	21.91	0.69	0.79	1.48	5270	10400

Table A.3 The kinetic rate law equations:

$\frac{dq_t}{dt} = k(q_c - q_t)^2$

k_2 is the second order rate constant of sorption (g mg^{-1} min^{-1})

q_c is the amount of soluted sorbate sorbed at equilibrium (mg g^{-1})

q_t is the amount of solute sorbate on the surface of the sorbent at time t (mg g^{-1})

h is the initial rate of adsorption (mg kg^{-1})

This can be rewritten as:

$$\frac{dq_t}{(q_c - q_t)^2} = k \cdot dt$$

Integrating for boundary conditions where t=0 to t=1 and q_t=0 to q_t=q_t

Further simplified leads to the integrated rate law for the pseudo-second order reaction.

$$\frac{1}{q_c - q_t} = \frac{1}{q_c} + kt$$

The above equation can be rearranged and simplified,

$$q_c - q_t = \frac{1}{\frac{1}{q_c} + kt} = \frac{q_c}{1 + q_c kt}$$

$$q_t = q_c - \frac{q_c}{1 + q_c kt}$$

$$q_c \left[1 - \frac{1}{1 + q_c kt}\right] = q_c \left[\frac{1 + q_c kt - 1}{1 + q_c kt}\right] = \frac{{q_c}^2 kt}{1 + q_c kt}$$

$$\frac{1}{q_t} = \frac{1 + q_c kt}{{q_c}^2 kt}$$

$$\frac{t}{q_t} = \frac{1 + q_c kt}{{q_c}^2 k} = \frac{1}{{q_c}^2 k} + \frac{t}{q_c} = \frac{1}{h} + \frac{1}{q_c} t$$

$$\frac{t}{q_t} = \frac{1}{h} + \frac{1}{q_c} t$$

$$y = \frac{t}{q_t}$$

To summarize:

Non-linear form: $q_t = \frac{{q_e}^2 kt}{1+q_e t}$

Linearized form: $\frac{t}{q_t} = \frac{1}{k_2 {q_e}^2} + \frac{1}{q_e} t$

Plots: $\frac{t}{q_t}\ vs\ t$

Slope: $\frac{1}{q_e}$

Intercept: $\frac{1}{k_2 {q_e}^2}$

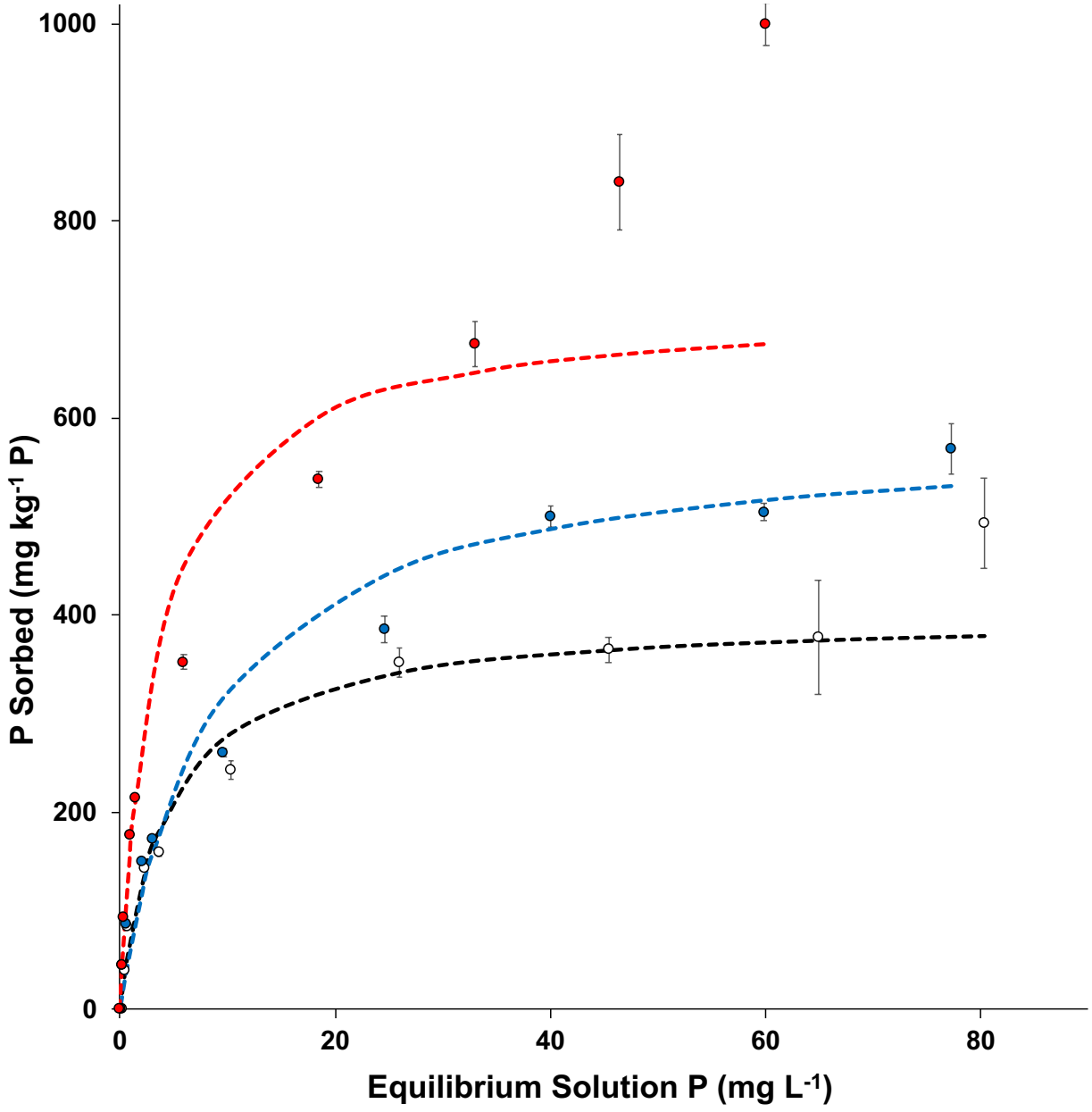

Figure A.3. Adsorption isotherm with Freundlich model

Table A.4. Langmuir and Freundlich model outputs

Soil	Langmuir		
	Qmax (mg kg-1)	b (1 kg-1)	R2
Control	379.2	0.0025	0.99
Depleted	531.6	0.0017	0.98
Saturated	674.9	0.0014	0.98

Soil	Freundlich		
	Qmax (mg kg^{-1})	b (1 kg^{-1})	R^2
Control	500.6	0.4035	0.94
Depleted	616.2	0.4131	0.99
Saturated	1121.8	0.4618	0.96

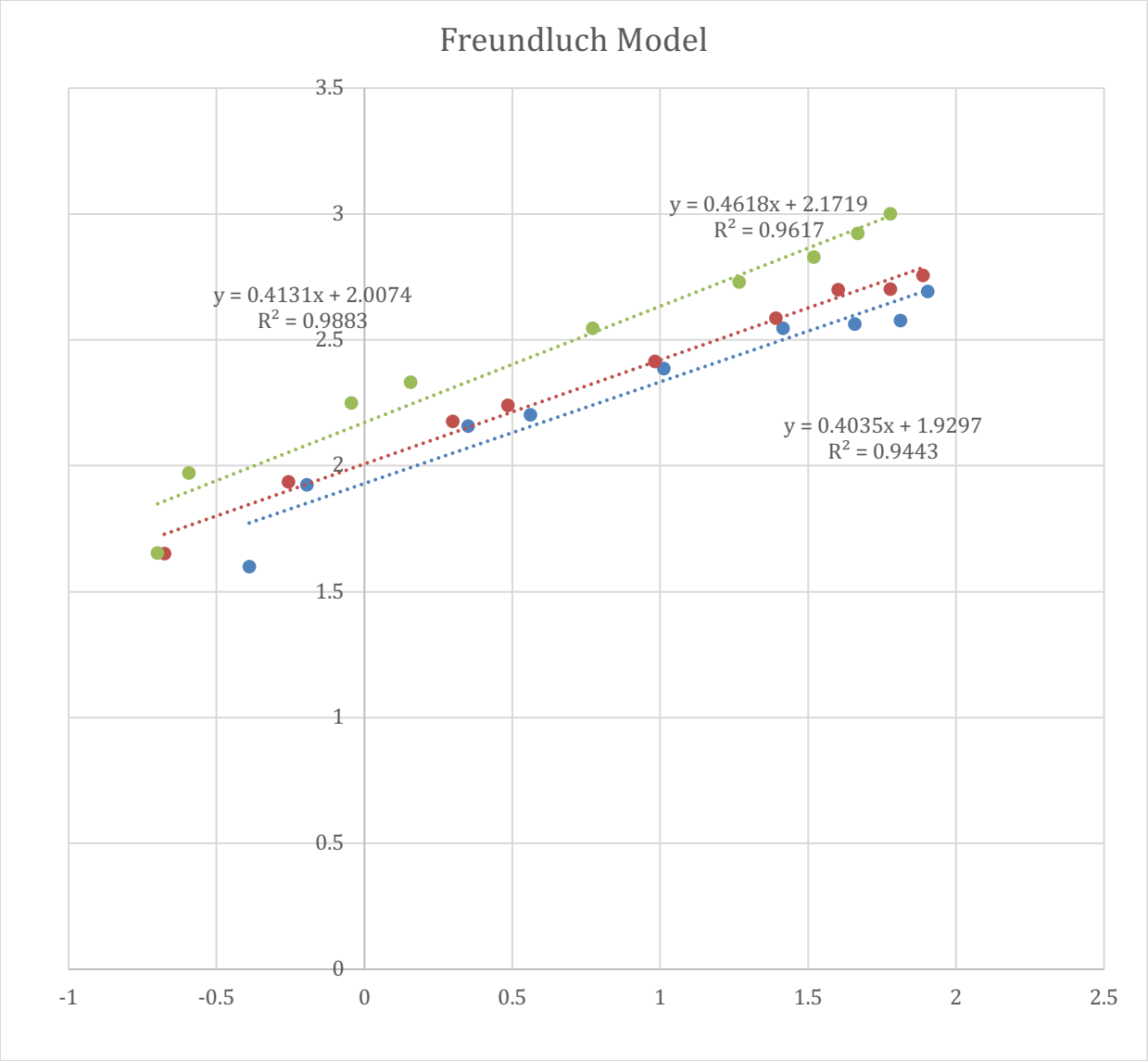

Figure A.4. Freundlich model graphed between Control, Ca saturated and depleted soils.